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BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE

БЮЛЛЕТЕНЬ ЭКСПЕРИМЕНТАЛЬНОЙ БИОЛОГИИ И МЕДИЦИНЫ

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BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE

VOLUME 45, NUMBER 6 JUNE ,1958

CONTENTS

	PAGE	RUSS. PAGE
Physiology		
Certain Data on the Character of Representation of Internal Organs in the Cerebral Cortex. N. V. Bratus'	649	3
Changes in the Electrical Activity of the Human Brain During the Initial Period of Muscular Activity. E. A. Mukhamedova	653	7
Electrophysiologic Investigation of the Mechanism of Chemoreception. Communication III. The Effect of Nicotine and Acids on the Intestinal Receptors in Monoiodoacetic- Acid-Induced Disturbances of Metabolism. N. A. Anikina	656	11
Reflex Changes in the Activity of the Cardiovascular System Under the Influence of Chemical Stimulation of Pericardial Receptors. Communication I. The Dependence of the Character of Reflex Reactions of the Cardiovascular System on the Intensity of Chemical Stimulation of Pericardial Receptors. B. S. Kulaev	662	17
Changes in Myocardial Trophics During the Chronotropic Effect. N. M. Abramova, V. F. Anisimova, A. V. Gutovskaia, A. V. Kibiakov, and Z. V. Urazaeva	667	22
The Role of Acetylcholine in the Development of Tetanic Single Response and Afterpotentials in the Nerve Trunk of Cold-Blooded Animals. G. I. Poletaev	670	25
Pathological Physiology and General Pathology		
Changes in the Duration of the Ventricular Complex of the Electrocardiogram During Interruption and Restoration of the Work of the Heart. D. G. Maksimov	674	30
The Effect of Long-Standing Experimental Arteriovenous Aneurysms on the Heart. L. A. Lomanov	680	36
The Role of the Hematoencephalic Barrier in the Pathogenesis and Therapy of Acute Cerebrospinal Trauma. B. I. Kamenetskaia	685	41
The Effect of Partial and Total Extirpation of the Cerebral Cortex on the "Mechanical Secretion" of Gastric Juice. A. S. Chechulin	688	44
The Study by the Method of Vital Staining of the Sorptive Properties of Certain Organs in Experimental Tuberculosis. Communication II. Investigation of the Sorptive Properties of Skeletal Muscle. M. V. Iakovlev	693	49
The Selective Effect of Some Chemical Stimulants on the Reflexes from Chemoreceptors. Communication VIII. The Effect of Streptomycin on Reflexes from the Chemoreceptors and Mechanoreceptors of the Pericardium. G. S. Kan	696	53
Comparison of the Active Concentrations of Some Antibiotics in the Lymph of the Peripheral Vessels and the Thoracic Duct. G. A. Ivanova	701	57

(continued)

CONTENTS (continued)

	PAGE	RUSS. PAGE
Biochemistry and Biophysics		
Interconversion of Liver and Serum Proteins in Homogenates of Rats' Liver. O. B. Kuzovleva	705	62
The Use of Mitogenetic Spectral Analysis for Detection of Acetylcholine in Frog's Muscle During Various Types of Stimulation. M. A. Lipkind	708	65
Pharmacology		
The Effect of Papaverine and Dibazol on the Rate of the Coronary Circulation. I. E. Kisin	712	69
Microbiology and Immunity		
The Relation of the Site of Action of the Antigen in the Body and the Site of Antibody Formation (In Experimental Immunization with Tetanus Toxoid). Communication III. A Comparison of the Value of Intramuscular and Intravenous Injection of Toxoid and the Importance in Immunization of Antigen Circulating in the Blood. L. N. Fontalin.	716	73
The Use of the Reaction of Increase of Phage Titer in the Detection of Flexner Dysentery Bacteria in Infected Rabbits. A. Iu. Illiutovich, Z. S. Petrova, E. E. Golubeva, and R. S. Chetvernina	721	78
Oncology		
The Significance of Higher Nervous Activity in the Appearance and Development of Tumors of the Breast in Mice. L. L. Maliugina, A. I. Mironova, V. K. Fedorov, and L. M. Shabad	727	85
The Leukemia-Producing Activity of Cell-Free Filtrates of Human Leukemic Tissue. V. M. Bergol'ts	731	89
Experimental Biology		
A Study of the Proliferative Properties of Corneal Tissues During Wound Healing. V. F. Pcheliakov	735	92
Analysis of the Mechanism of Degradational Mitogenetic Radiation. V. F. Eremeev	739	95
Regeneration of Supernumerary Limbs in Tadpoles. A. A. Voitkevich	744	100
A Trial of Growth Hormone Preparations in Rats Whose Thyroid Function Has Been Blocked with Methylthiouracil. N. K. Demokidova	747	104
Methods		
Recording the Action Potentials of Nerves in An Unscreened Room. L. A. Osterman	751	109
A Method of Studying Gastric and Duodenal Secretion at the Same Time. V. E. Robinson and F. I. Mizgirev	754	112
Experimental Heterotransfusional Shock in White Rats Accompanied by Acute Pulmonary Edema. G. V. Kurygin	757	114

PHYSIOLOGY

CERTAIN DATA ON THE CHARACTER OF REPRESENTATION OF INTERNAL ORGANS IN THE CEREBRAL CORTEX

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(Received November 9, 1956. Presented by Active Member Acad. Med. Sci. USSR, V. V. Parin)

The presence of a connection between the internal organs and the higher levels of the central nervous system has been demonstrated convincingly by the method of conditioned reflexes. However, the question of the character of this connection cannot be considered as finally settled.

There is a view according to which the cerebral end of the internal analysor is unlikely to be represented in the form of "specially delimited, strictly localized areas" [1]. At the same time electrophysiologic investigations have established that stimulation of the internal organs (reflected in the bioelectric activity of the whole cerebral cortex) elicits more intense, qualitatively different changes in definite areas of the cortex, viz. in the premotor region [2, 3, 5, 7, 11]. An important advance in the study of the structure of the visceral analysor is the investigation of primary responses to stimulation of visceral nerves [14, 17]. Foreign workers have established by this method the projection of the splanchnic nerves to the sigmoid and anterior ectosylvian gyri. Representation of the pelvic nerve has been demonstrated in approximately the same region in the laboratory of V. N. Chernigovskii [12]. Earlier, stimulation of the vagus was found to be accompanied by increased oscillations of potential in the orbital region of the frontal lobes [16].

Elucidation of the projection in the cerebral cortex of large collectors of interoceptors gives a definite picture of the structure of the cerebral end of the visceral analysor. An important stage is represented by studies of the distribution of receptive zones in the cerebral cortex as shown by stimulation of separate internal organs [8].

The present work is concerned with the study of the bioelectric activity of the cerebral cortex on stimulation of gastric receptors.

EXPERIMENTAL METHOD

Experiments were carried out on 25 dogs under short-term experimental conditions. The preliminary operation was performed under ether-morphine anesthesia. It consisted of introduction of a rubber balloon into the stomach and trephining of the skull with removal of the dura.

Stimulation was effected by inflation of the balloon introduced into the stomach; air bellows were used and the pressure obtained was equal to 30 mm Hg. Cerebral biopotentials were recorded with a cathode ray oscillograph with a rheostat-capacity amplifier. Amplification was such that a voltage of 100 μv at the amplifier input produced a 4 mm deflection of the oscillograph beam. The image was reduced by half when the record was photographed. The recording electrodes were made of digestive tin.* The indifferent electrode, in the form of a rod with thread cutting, was screwed into the nasal bone. The active electrode was a loop on which was wound a fine wick of cotton moistened with physiologic solution.

The experiments were started 1-1.5 hrs after the preliminary operation. The animal was placed in a darkened, screened and sufficiently sound-proof chamber. The investigations were carried out under deep urethan or ether anesthesia. Urethan was given intravenously in the amount of 1 g per kg of body weight.

* Literally, "alimentary nutritive"; probably tin which can be left in brain and not cause damage. Editor.

EXPERIMENTAL RESULTS

When the gastric mechanoreceptors were stimulated by a single inflation, the cerebral cortex of the anesthetized animal showed a peculiar localized reaction. It was expressed in most cases by a diphasic wave in which the first phase is positive and the second negative. In some of the experiments, especially on deepening the anesthesia, the reaction to such stimulation manifested itself by a monophasic wave, more often positive than negative. The area from which this reaction was recorded corresponded to the posterior part of the sigmoid gyrus, being approximately half way between the anterior and posterior cruciate sulci. The amplitude of the biopotentials varied greatly and fluctuated between 100 and 1000μv, changing both from experiment to experiment and in the same animal. The duration of the electric reaction was from 40 to 100 msec in different experiments.

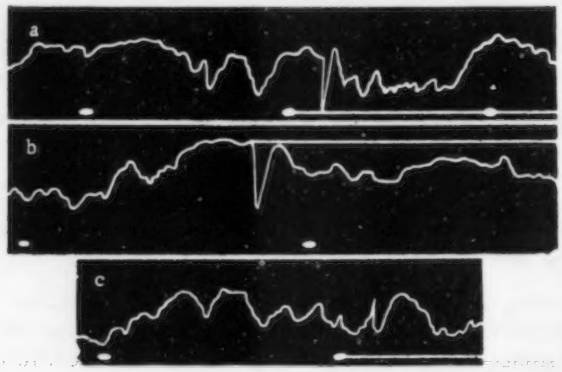

Fig. 1. Electrocorticogram of dog recorded from g. cruciatus posterior dextra under deep ether anesthesia. Experiment 15, October 1956. a) Recording electrode placed on a point of cortex lying approximately midway between the anterior and posterior cruciate sulci; period of stimulation of the stomach (pressure 30 mm Hg) marked by solid line; b) recording electrode placed in the same area of cortex; solid line marks stimulation of the gastric nerve branch (central part) by single induction shock; c) recording electrode moved 1 mm towards the posterior cruciate sulcus, recording reaction to gastric stimulation. Time marker – 1 sec.

Figure 1,a shows the electrocorticogram from the right posterior cruciate gyrus in dog under ether anesthia. Mechanical stimulation of the stomach elicits a diphasic action potential with a positive phase followed by a negative one. The amplitude of the first phase is 400 μv, of the second 150 μv. The duration of this reaction is 80 msec. In a number of its aspects (configuration, amplitude, sequency of phases, localization) such a reaction resembles the primary response observed on stimulation of exteroceptors.

Additional support for the probability of such a suggestion was afforded by investigations in which the gastric nerves were subjected to electric stimulation. In response to stimulation by single induction shocks the electric reaction appeared at the same cortical points as those from which the bioelectric effect was recorded after stimulation of the stomach (Fig. 1, b; 2,b). Both reactions showed certain similarities with respect to the form of the biopotentials. The main difference between the bioelectric effects obtained on stimulation of the nerves and receptors of the stomach is the difference in the duration of the latent period, which is shorter when the nerve is stimulated. Our method does not allow precise determination of the true extent of the latent period following stimulation of the stomach, since in these experiments only the beginning of inflation of the balloon was recorded and not its complete inflation.

The extent of the cortical area from which localized bioelectric response to stimulation of the stomach can be obtained is about 3 mm. When the electrode is moved from the main points toward the periphery there is a change in the character and amplitude of the biopotentials (Fig. 1,c). At more distant cortical areas the stimulation only elicits a change in the basic rhythm of electrical activity.

Our experiments have shown that a localized cortical response may arise not only at the beginning of gastric stimulation but also after its cessation. Direct proof of this was obtained in the electrophysiologic investigations of V. E. Delov and his collaborators [6] who found impulses in the gastric nerves both during distention and collapse of the gastric wall.

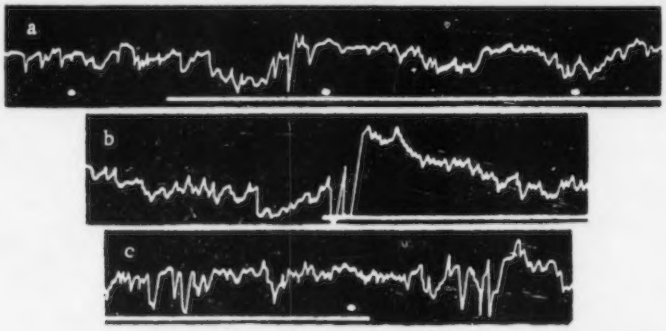

Fig. 2. Electrocorticogram of dog recorded from g. curciatus posterior dextra. Experiment June 30, 1956. a) On stimulation of the stomach under deep ether anesthesia; b) on stimulation of the gastric nerve branch by induction current; c) on stimulation of the stomach and after its cessation under lighter anesthesia. Time marker – 1 sec. Horizontal line on traces – stimulus marker.

When excitability is increased as, for example, in lighter anesthesia, stimulation of the stomach may elicit a series of electric complexes in the projection area of the cerebral cortex which follow each other in a train both at the moment of stimulation and for some time after cessation of stimulation (Fig. 2,c). This phenomenon has been described by a number of authors in connection with stimulation of exteroceptors [10, 13, 15] or visceral nerves [14] and is interpreted as an expression of excitation of thalamocortical systems by impulses arriving from the periphery [4]. On this basis, the repetitive discharges in our experiments can be explained by the arrival of impulses in the brain from gastric receptors. However, this question requires special elucidation. When analyzing the bioelectric reaction it is important to solve the problem, in principle, concerning the origin of this reaction: could it be a primary response or is it a secondary cortical response ? It is known that the main differences between the primary and secondary responses lie in the difference of the duration of the latent period (which is markedly longer in the case of secondary responses) and in the spatial extent of the reaction in the cortex – secondary responses can be recorded from almost the whole of the cerebral cortex whereas primary responses are observed only at strictly localized cortical areas.

Since experiments with stimulation of the gastric nerve branches gave records of bioelectric phenomena similar to those found on stimulation of the stomach but with considerably shorter latent periods, it is possible to regard the reaction as a primary response. The main basis, however, for such a conclusion is the clearly localized nature of the reaction both to stimulation of the stomach and of the gastric nerves.

The somewhat more prolonged character of the reaction in our experiments as compared with the data of other authors must be explained by the special features of the experiments. Mechanical stimulation of the stomach is unlikely to be associated with a synchronous volley of impulses at the cerebral cortex. A series of impulses proceeding from a mass of mechanoreceptors in the stomach can elicit a more prolonged cortical bioelectric effect. There are references in the literature to prolongation of the primary effect on stimulation of exteroceptors [10].

A clear primary response is obtained on stimulation of the stomach under deep anesthesia (in our experiments with urethan or ether) when the level of "spontaneous" electrical activity of the brain is significantly lowered but the excitability of the cortical cells with respect to interoceptor impulses does not disappear.

Beyond the limits of the area from which the primary response is recorded, upon stimulation of the stomach a so-called generalized reaction develops which manifests itself in changes in the character of the basic electrical activity. Our data obtained by electrophysiologic methods serve as yet another proof of I. P. Pavlov's hypothesis [9] that "stimulation arising in receptors impinges upon certain definite points of the cerebral cortex."

SUMMARY

Short-term experiments were performed on dogs under ether or urethan anesthesia. It was established that a localized bioelectric reaction appeared in the area of g. cruciatum posterior as a result of stimulation of the stomach mechanoreceptors or of electric stimulation of the gastric nerve branches. This reaction may be considered as a primary response. Data on the primary response following stimulation of the stomach may be used for determination of the projection of the stomach receptor fields in the cerebral cortex.

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* In Russian.

** Russian translation.

CHANGES IN THE ELECTRICAL ACTIVITY OF THE HUMAN BRAIN DURING THE INITIAL PERIOD OF MUSCULAR ACTIVITY

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(Received April 29, 1957. Presented by Active Member Acad. Med. Sci. USSR, V. V. Parin)

The number of investigations dealing with the electric activity of the brain during muscular activity is very limited owing to practical difficulties. There has been no special study of changes in electrical activity of the brain during the initial period of work, although some authors have noted that the beginning of muscular activity was accompanied by the appearance of fast activity in the electroencephalogram of the same type as muscle potentials [5] or by depression of the α-rhythm [4].

The aim of the present work was the systematic study of changes in the electrical activity of the brain during the initial period of muscular activity.

EXPERIMENTAL METHOD

The electroencephalogram was recorded during the performance of work by the method described earlier [2]. Reliable contact between the electrode and the scalp was achieved by careful application of stick-on electrodes to the muscle-free parts of the scalp; paste and additional "bracing" of the leads were employed. Standard positions of the electrodes were determined experimentally.

The electrical activity of the brain was recorded by means of a 4-channel ink-writing electroencephalograph (made at the AMN SSSR experimental plant). Bipolar recording was usually used. The movements investigated in this work were familiar and habitual for the subject: work on bicycle-ergometer of moderate power (15-50 revolutions of the pedals per minute), various types of rhythmic hand movements with different loads, static muscle exertions (trunk, arms, legs). A total of 80 experiments were carried out on 7 subjects. At rest all the subjects showed an α-rhythm and the response to eye opening and eye closure was of the usual kind.

EXPERIMENTAL RESULTS

The beginning of muscular activity gave rise to depression of the α-rhythm in the electroencephalogram during which most of the subjects showed activity of the β-rhythm type. As the work proceeded small groups of α-waves, alternating with periods of α-rhythm depression, began to appear against this background. The frequency of the α-rhythm increased by 1-2 cps as compared with its frequency at rest; the amplitude was decreased. Sometimes some of the temporal leads showed slower activity at a frequency of 3-4 cps. Later, the bursts of α-rhythm became more definite and prolonged, while periods of its depression diminished in duration. The frequency of the α-rhythm itself decreased and the amplitude increased until the two returned to the values observed at rest. In some cases it was possible to discover a definite periodicity in the appearance of the bursts of α-waves, coinciding with the rhythm of the movement.

Further continuation of work was accompanied by α-rhythm which was not significantly different from α-rhythm at rest.

The time which elapsed from the beginning of work until the appearance of α-rhythm with frequency and amplitude characteristic for the resting state was taken as the duration of the initial period of work. Figure 1 shows the initial period of muscular activity upon flexion and extension of the arm on an ergograph with a load of 1.5 kg. The beginning of the familiar muscular activity was accompanied by depression of the α-rhythm and the appearance of fast activity of β-rhythm type in the encephalogram; as the work continued, the fast activity was gradually replaced by α-rhythm.

In this experiment muscle potentials were recorded from the active muscles simultaneously with the EEG. The electromyogram shows that as work continues the duration of the bursts of muscle potentials decreases and becomes stabilized, which denotes gradual perfection of the movements performed as compared with the initial period [1].

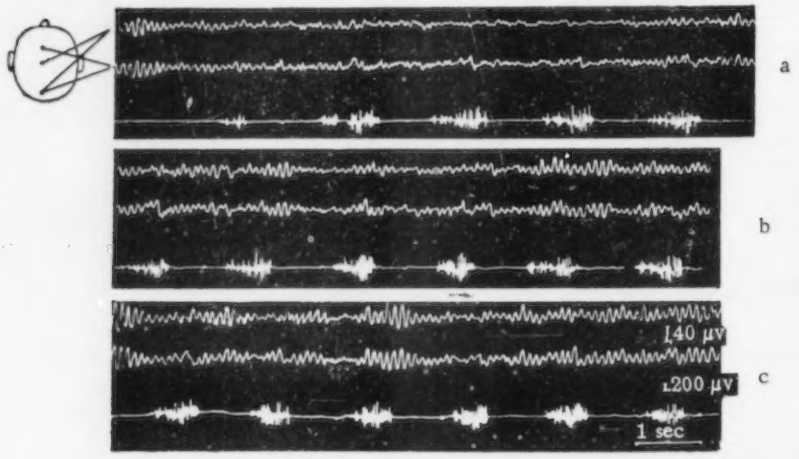

Fig. 1. Changes in the electrical activity of the brain in subject G. during performance of work. During the initial moment of muscular activity the EEG shows depression of the α-rhythm. On continuation of work there is restoration of the α-rhythm to its resting characteristics. Sections a, b, c are a continuous record of the same EEG, c) recorded 1 min 30 sec after the beginning of work. The lower trace in each section – electromyogram; bursts of muscle potentials correspond to the rhythm of the movement.

The EEG changes described above as characterizing the beginning of work were not always sufficiently clear. The degree of change of the EEG components and the duration of the initial period of work could show considerable fluctuations. Even in one and the same experiment the changes in electrical activity of the brain during the beginning of work were not constant but depended on the character and power of the work performed earlier and subsequently in that particular experiment.

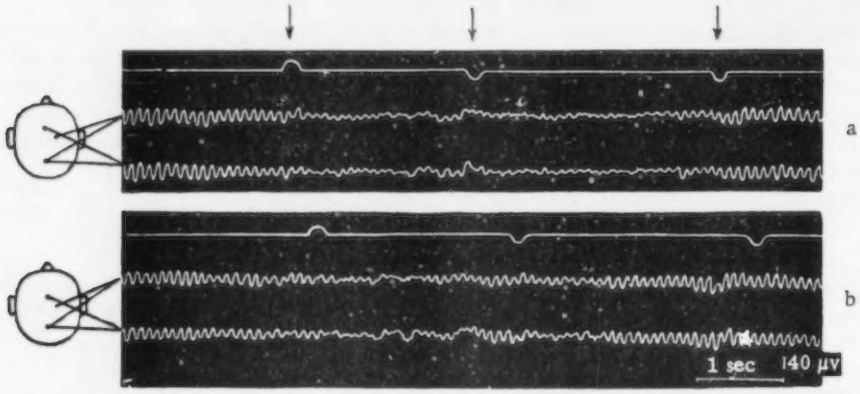

Fig. 2. Changes in the electrical activity of the brain in subject K-o on repeating the work. Diminution of the initial period is noted. a) First performance of the work; b) repeat of performance of the work; ↓ – signal denoting beginning of movement; upper tracing – recording of the rhythm of movement.

Repeating work of the same type and degree reduced the initial period of the work. This was observed on repeating the same type of muscular activity from one experimental day to another and even more so on repeating the work after short intervals of time (2-5 min). Figure 2 shows that a repetition (after 2 min) of rhythmic movements – elevation and lowering of the arm with a load – is accompanied by smaller changes in the EEG and a shorter initial period of work which diminished from 5 sec during the first performance of the work to 3 sec when it was repeated.

Preparatory work, carried out with greater effort (higher frequency of movement) decreased the initial period of subsequent work. When the preparatory work was different in coordination pattern from the subsequent work the initial period was longer and the EEG changes greater than in cases in which the preparatory work was similar in nature.

For example, after work consisting of the same movements (rhythmic elevation and lowering of the arm) the initial period was equal to 6 sec. After complex (from the point of view of coordination) exercises performed simultaneously by the left arm and leg, the initial period of subsequent rhythmic movements of the arm increased to 9 sec and the depression of the α-rhythm was more profound and prolonged.

By specially selecting the exercises for preparatory and subsequent work, varying their frequency, number of repetitions, etc., it is possible to achieve diminution of the initial period of work and even its disappearance. In the latter case the EEG will show, from the very beginning of muscular activity, a stable α-rhythm, as shown in Fig. 3.

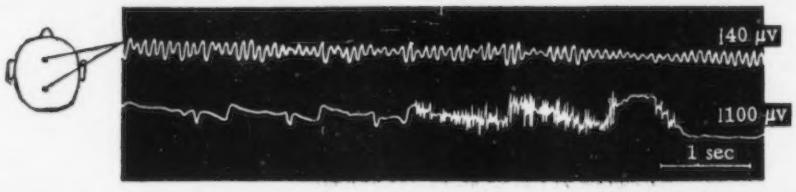

Fig. 3. Absence of changes in the electrical activity of the brain in the subject K-o during the beginning of work. Records (from above down): electroencophalogram, biceps electromyogram on elevation of the load.

The adaptation of the organism to new conditions is associated with certain "tension" – certain adjustment. "Time is required to achieve a new adjustment" wrote I. P. Pavlov [3].

The process is reflected also in the electrical activity of the brain: at the beginning of work there is depression of the α-rhythm with the appearance of β-rhythm-type of activity which becomes replaced by groups of faster α-waves as the work continues; the EEG then shows a stable α-rhythm with frequency and amplitude similar to those observed in the resting state. Evidently this reflects the interrelations of the main nervous processes in the cortex of the cerebral hemispheres. It may be postulated that during the beginning of work there is a change in the balance of the main nervous processes towards predominance of excitation in certain groups of cortical cells which probably finds reflection in depression of the α-rhythm and the appearance of the β-rhythm type of activity. Further repetition of the same movements apparently requires less "tension" of the excitatory process as shown by the constant restoration of the α-rhythm with decrease in its frequency and increase in its amplitude and the appearance of waves slower than the α-rhythm. The periodic appearance and disappearance of groups of α-waves evidently denotes the existence of "uninterrupted struggle between excitation and inhibition" (I. P. Pavlov).

As the movements become completely automatic an α-rhythm indistinguishable from the resting α-rhythm is recorded.

The initial period of muscular activity is very reactive: the depth of changes in the EEG and the duration of this period may alter significantly (up to complete disappearance) depending on the number of repetitions of the same type of activity and on the type and degree of the preparatory work. Such sensitive reaction suggests that alteration of the type of muscular activity may hinder or facilitate the adjustment of the organism to the work it is to be required to perform.

SUMMARY

Initial muscular activity is associated with depression of the α-rhythm and appearance of high-frequency oscillations of the β-rhythm type in the electroencephalogram. On continuation of the work, short periods of α-rhythm begin to appear on this backgroun and gradually increase. If the work is continued further it is accompanied by an α-rhythm which is almost no different from the α-rhythm in the state of rest. The duration of the initial period of work depends on the character and power of the preparatory and the subsequent work.

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ELECTROPHYSIOLOGIC INVESTIGATION OF THE MECHANISM OF CHEMORECEPTION

COMMUNICATION III. THE EFFECT OF NICOTINE AND ACIDS ON THE INTESTINAL RECEPTORS IN MONOIODOACETIC-ACID-INDUCED DISTURBANCES OF METABOLISM

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Many authors are at present concerned with the problems of chemoreception [11]. There is still no single viewpoint, however, with respect to the mechanism of the action of various chemical stimuli. The majority of authors divide stimuli into 2 groups: the first includes hypercapnia and hypoxia as well as cyanide compounds, the second acetylcholine, nicotine and some others.

Many authors, in their attempts to approach the solution of the essential problem of chemoreception, chose the path of studying changes in metabolic processes which play an important part in the reception of the stimuli indicated [12, 9, 3].

It has been suggested [9] that the stimulus for chemoreceptors is a change of metabolism within the cells. The author supposed that there were two different mechanisms of chemical stimulus action on receptors. Substances belonging to the first group cause significant changes in cell metabolism which serve as chemoreceptor stimuli.

* In Russian.

Stimuli belonging to the second group act directly on receptors and possibly produce no marked changes in tissue cell metabolism. Such a concept is supported by experiments [9, 10, 8] in which it was shown that after chemoreceptors had been acted upon by certain substances (sodium fluoride, monoiodoacetic acid), carbon dioxide and a number of other substances produced none of the characteristic changes in blood pressure and respiration; at the same time, reflexes elicited by nicotine and acetylcholine were preserved. Moreover, following the action of monoiodoacetic acid, the reactions to carbon dioxide at first disappeared and then showed inversion from pressor to depressor, while the the reactions to nicotine were enhanced [5, 7].

In our experiments [2] we showed that when intestinal tissue metabolism was altered by the action of monoiodoacetic acid (4-5 ml 0.01 N solution), administration of nicotine produced a greater flow of impulses than in the normal and a higher rise of blood pressure. Carbonic acid caused a depressor blood pressure reaction and inhibition of initial impulse flow instead of its enhancement,as was observed before monoiodoacetic acid treatment. These data also supported the difference of monoiodoacetic acid (MIA) effect on nicotine and carbon dioxide reception.

However, according to M. L. Belen'kii's data [3], the reception of both groups of chemical stimuli is impaired after treatment of carotid body chemoreceptors with monoiodoacetic acid and other poisons which block various stages of carbohydrate metabolism. This author studied the effect of cyanides and lactic acid, as well as acetylcholine. He therefore considers that reception of various groups of chemical stimuli is connected with the same links in carbohydrate metabolism.

We have postulated the view that the different results obtained by the authors cited [9, 10, 8 and 3] depend to a considerable degree on the use of different doses of enzyme poisons in their experiments.

Moreover, in some experiments [3] the action of monoiodoacetic acid on the chemoreceptors was effected by means of prolonged perfusion whereas in others [8, 9, 10] the effect of a single administration of a relatively small amount of monoiodoacetic acid was studied.

The aim of the present investigation was the study of reception of various groups of chemical stimuli under conditions of impaired intestinal tissue metabolism effected by different doses of MIA. The receptor reaction under the influence of various doses of monoiodoacetic acid was judged on the basis of reflex changes in blood pressure arising from the action of chemical stimuli on intestinal receptors, and of changes in afferent impulse flow in intestinal nerves.

EXPERIMENTAL METHOD

Experiments were carried out with perfusion of an isolated portion of the intestine connected with the organism by nerves only. The chemical stimuli – nicotine and acids – were introduced into the perfusion fluid. Afferent impulses in the intestinal nerves were recorded simultaneously with blood pressure in the carotid artery (the method is described in detail in previous communications [1, 2]). The action of nicotine and various acids (carbonic, acetic and lactic) on intestinal receptors was studied afterthe use of different doses of monoiodoacetic acid. The influence of monoiodoacetic acid was studied both on single administration of its solutions by way of the perfusing fluid and on prolonged perfusion of fluid containing monoiodoacetic acid through the intestinal vessels. A total of 30 experiments was performed.

EXPERIMENTAL RESULTS

In the first experiments we introduced single relatively large doses of MIA which were larger than those used previously [8, 10, 9].

The data obtained are presented in Fig. 1 which shows that after introduction of 5 ml monoiodoacetic acid (0.01 N solution) there was increased intensity of impulses in response to administration of nicotine as compared with the effect obtained prior to the action of MIA (see Fig. 1, II and III). Subsequent administration of a relatively large amount of MIA (1 ml 0.1 N solution) resulted in the disappearance of impulses in response to subsequent administration of nicotine (Fig. 1, IV). Changes in blood pressure in response to nicotine were also absent under these conditions.

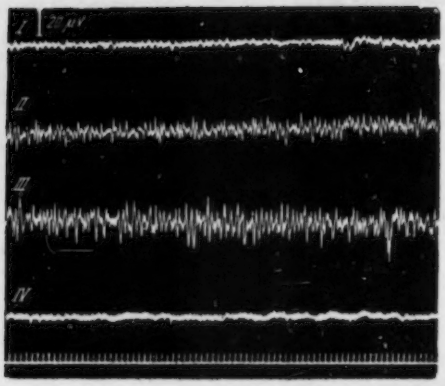

Fig. 1. Changes in intestinal receptor response to the action of nicotine after introduction of various doses of monoiodoacetic acid into the intestinal vessels. I) Initial electric activity in intestinal nerves; II) electric activity after introduction of 1 ml nicotine in 10^{-4} concentration into the intestinal vessels; III) the same with nicotine concentration 10^{-4} 10 min after introduction of 5 ml 0.01 N solution of monoiodoacetic acid into intestinal vessels; IV) the same with concentration of nicotine 10^{-4} after second introduction of monoiodoacetic acid (1 ml 0.1 N solution). Time marker 0.02 sec.

Thus in experiments with introduction of large amounts of monoiodoacetic acid, the effect of nicotine underwent changes similar to those in the experiments of M. L. Belen'kii [3]. However, the disappearance of the reaction to nicotine under the conditions indicated may not signify a specific interference of monoiodoacetic acid with metabolic processes participating in nicotine uptake, since introduction of a single large dose of this enzyme poison may produce irreversible "extinction" of receptors.

It was therefore essential to find the conditions under which it would be possible to trace the dynamics of changes in afferent impulse flow and reflex changes of blood pressure elicited by various stimuli acting on the intestinal receptors against a background of a gradually increasing influence of MIA. In connection with this, subsequent experiments were performed with the effect of nicotine studied against continuous perfusion of intestinal vessels with monoiodoacetic acid solution (0.002 N). In these experiments, changes of reaction to various stimuli appeared relatively rapidly. The experiments showed that reaction to all the acids studied was the same prior to the action of monoiodoacetic acid, during its action and after it had been washed away.

When a series of reflexes to chemical stimuli had been recorded, a solution of monoiodoacetic acid was passed through the vessels and against this background the reflexes and afferent impulses under the action of the indicated chemical stimuli were studied again. Following this perfusion with Ringer-Locke solution was resumed.

Figure 2 (1-3) shows pressor reactions of the blood pressure arising on introduction of 0.5 ml 2% lactic acid, 5 and 50 γ nicotine into the intestinal vessels. After this, perfusion with a solution of monoiodoacetic acid (0.002 N) was begun. At first there was a decrease and then disappearance of the reaction to nicotine in the dose 5γ (see Fig. 2: 4,5), then to lactic acid (Fig. 2: 6, 8), and finally to nicotine in the dose 50γ (Fig. 2: 9). Following the disappearance of the reaction to nicotine in the dose 50γ, perfusion with monoiodoacetic acid was terminated and perfusion with pure Ringer-Locke solution was resumed.

Figure 2 (10) shows that after this pressor reaction in response to nicotine in the dose 50γ are restored and then to nicotine in the dose 5γ [Fig. 2: 12], the latter reaction being even somewhat greater than that observed prior to the action of monoiodoacetic acid. The reaction to lactic acid, however, is first absent (Fig. 2: 11) although in the initial state it was considerably greater than the reaction to nicotine in the dose 5γ.

During perfusion with Ringer-Locke solution, the blood pressure reaction to administration of lactic acid becomes weakly depressor (Fig. 2: 13). On further perfusion with Ringer-Locke solution the depressor reaction to lactic acid action becomes more marked up to a certain moment, while the reaction to nicotine increases to a level exceeding the initial one.

Similar results were observed in all our experiments with the use of the monoiodoacetic acid concentration indicated.

Figure 3 (2, 3) shows the changes in afferent impulses under the influence of lactic acid and nicotine before perfusion with MIA; Fig. 3 (5, 6) shows corresponding changes in impulses against the background of MIA perfusion; Fig. 3 (8, 9) shows the impulses after washing with Ringer-Locke solution; Fig. 3 (1, 4, 7) shows the records of "background" impulses. It can be clearly seen from Fig. 3 (2, 3) that slow impulses arise in response to the action of both nicotine and lactic acid. The appearance of impulses coincides with the appearance of pressor reactions of the blood pressure as the result of the action of these stimuli. As can be seen from Fig. 3

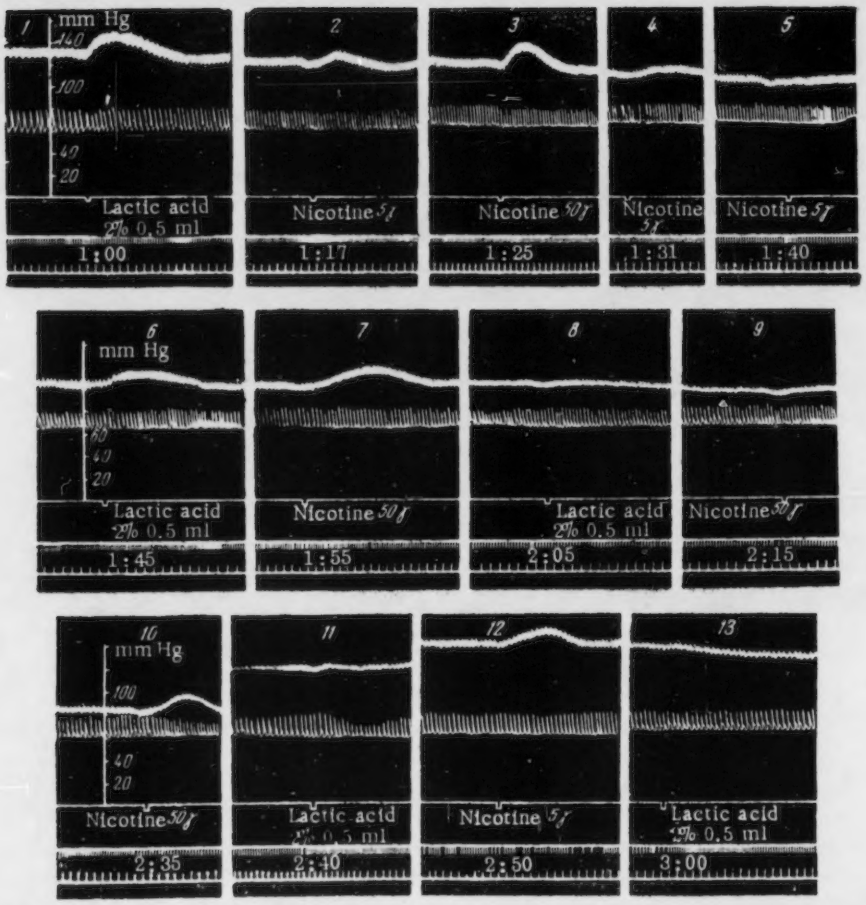

Fig. 2. Reflex changes in blood pressure under the action of nicotine and lactic acid on intestinal receptors before passage of monoiodoacetic acid (1-3) against background of perfusion with monoiodoacetic acid solution (4-9) and during washing with Ringer-Locke solution (10-13). Passage of monoiodoacetic acid was begun at 13 hrs 29 min and terminated at 14 hrs 17 min. Records (from above down): blood pressure in carotid artery, respiration, marker denoting administration of stimulus, record of the rate of perfusion, time marker (5 sec).

(5, 6) no impulses arise in response to the same stimuli, which coincides with complete absence of blood pressure reaction. Slow impulse activity again appears under the influence of nicotine as can be seen from Fig. 3 (8, 9). This coincides with the moment of restoration of pressor reactions to nicotine. In response to the action of lactic acid (Fig. 3: 8) there is, on the contrary, a small inhibition of "background" activity (Fig. 3: 7) which coincides with development of a depressor reaction of the blood pressure.

Deepening action of monoiodoacetic acid was associated with cessation of afferent-impulse enhancement and of reflex changes in blood pressure as the result of action of both groups of stimulating agents. However, when MIA perfusion of the intestine was terminated and washing with Ringer-Locke solution instituted instead, the reaction to nicotine was restored and this substance again elicited enhancement of impulse flow and reflex changes in blood pressure; the ability of acids to elicit impulse flow and pressor reactions of the blood pressure was, however, lost irreversibly. Several minutes after the beginning of Ringer-Locke solution perfusion, introduction of nicotine to the intestinal receptors caused increased blood pressure reaction, whereas the action of acid not only did not enhance the background afferent impulse flow, but inhibited it, and elicited depressor reactions of the blood pressure.

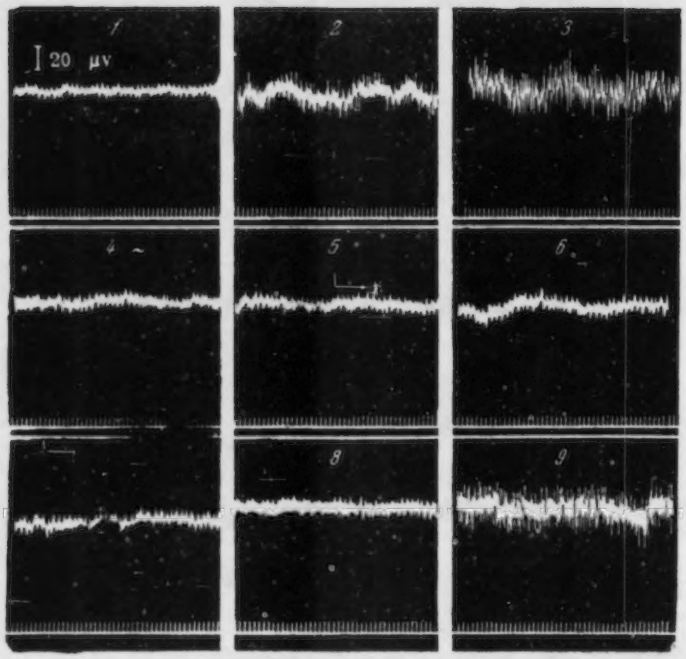

Fig. 3. Changes in afferent impulses in intestinal nerves under the action of nicotine and lactic acid on intestinal receptors before perfusion (2, 3) against the background of perfusion with monoiodoacetic acid (5, 6) and during washing with Ringer-Locke solution (8, 9). 1, 4, 7) Electric activity in intestinal nerves without stimulation; 2, 5, 8) changes in impulses under the influence of 0.5 ml 2% solution of lactic acid; 3, 6, 9) the same under the influence of 50γ nicotine. Time marker 0.02 sec.

Disappearance of the reaction to nicotine under the influence of large doses of MIA is, in our opinion, not the result of a specific blocking action of this substance on definite enzymatic processes connected with the mechanism of action of this stimulus, since these processes are disrupted irreversibly by MIA but the reactions to nicotine are restored.

How then can the transient disappearance of the reactions to nicotine be explained?

Experiments (6, 4, 2) have shown that prolonged action of carbon dioxide is associated with disappearance of reaction to all stimuli but, following washing with Ringer-Locke solution, the reflex returns. We carried out special experiments with perfusion of the intestine with a solution of acetic acid (0.1%); reflexes to both groups of stimulating agents disappeared very rapidly (2-5 min). On restoration, the blood pressure reactions to acid and to nicotine again assumed a pressor character. MIA also, evidently, can elicit temporary disappearance of the reaction to nicotine since it possesses acid properties.

The irreversible loss of intestinal receptor excitation in response to acids observed under the influence of MIA and expressed in the absence of a burst of impulses appearing in response to the action of the acids indicates that with respect to acids, monoiodoacetic acid behaves as an enzyme poison which blocks the metabolic processes participating in the reception of this group of stimuli.

Our experiments have thus confirmed our suggestion that the differences between the results obtained by V. N. Chernigovskii and M. L. Belen'kii are due to differences in the action of different doses of monoiodoacetic acid on receptors. By using doses of monoiodoacetic acid larger than those employed in V. N. Chernigovskii's experiments, we obtained, on the background of perfusion, results similar to those reported by M. L. Belen'kii.

However, the difference in intestinal receptor reaction to the 2 groups of stimuli found by us during the period of washing after MIA application, as well as the data previously obtained by V. N. Chernigovskii and V. A. Lebedeva [5-10], favor the concept of the existence of different mechanisms of action on receptors by different groups of chemical stimuli. It would appear that reception of nicotine and substances similar to it in their action does not include mechanisms which are blocked by monoiodoacetic acid whereas the uptake of acids by the receptors is impossible after specific blocking of these mechanisms.

The enhanced excitability of receptors with respect to nicotine and inhibition of these receptors with respect to acids observed during the period of washing after monoiodoacetic acid perfusion are evidently explained by the nonspecific influence of MIA on receptor excitability.

SUMMARY

The reception of various groups of chemical stimuli (nicotine and certain acids) was studied in conditions of deranged metabolism of intestinal tissue under the influence of various doses of monoiodoacetic acid. Experiments were performed with perfusion of an isolated portion of intestine connected to the organism by nerves alone. As the effect of monoiodoacetic acid becomes more pronounced the increase of the afferent impulse flow ceases and reflex changes of the blood pressure disappear.

Reactions of nicotine are re-established, the afferent flow increase and the reflex changes of the blood pressure recur after washing in Ringer-Locke's solution. At the same time, administration of acid depresses the impulse flow and causes depressive reactions of the blood pressure.

The conclusion was reached that the mechanisms blocked by monoiodoacetic acid evidently take no part in reception of nicotine and drugs with a similar effect. The reception of acids is impossible after specific block of these mechanisms.

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** In Russian.

REFLEX CHANGES IN THE ACTIVITY OF THE CARDIOVASCULAR SYSTEM UNDER THE INFLUENCE OF CHEMICAL STIMULATION OF PERICARDIAL RECEPTORS

COMMUNICATION I. THE DEPENDENCE OF THE CHARACTER OF REFLEX REACTIONS OF THE CARDIOVASCULAR SYSTEM ON THE INTENSITY OF CHEMICAL STIMULATION OF PERICARDIAL RECEPTORS

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V. N. Chernigovskii [14, 15] has established that stimulation of pericardial receptors in the rabbit with nicotine produces reflex fall of blood pressure and bradycardia, whereas under the influence of novocain a rise of blood pressure and acceleration of the heart rate take place. In recent years it became clear [17, 18] that stimulation of the reflexogenic zones of the thoracic cavity (heart, lungs) with veratrine leads to a sharp fall of blood pressure and bradycardia (the Bezold-Jarisch phenomenon). The work of V. N. Chernigovskii and his collaborators has shown that chemical stimulation of interoceptors in the abdominal viscera leads to reflex rise of blood pressure and acceleration of the heart, while exclusion of these interoceptors results in lowering of blood pressure and slowing of the heart [15, 16].

Faced with the question concerning the reasons for the differences between the influence of thoracic and abdominal reflexogenic zones on the cardiovascular system, we noted that the heart and the areas directly adjacent to it are particularly rich in sensory endings. In the heart itself, particularly in the ventricles, the largest number of receptors are concentrated in the epicardium and the endocardium [2, 6, 8, 9], while in the myocardium their number is relatively small; in the lungs the greatest number of receptors are in the mediastinal pleura which is directly adjacent to the pericardial sac [12, 2, 23]. The epicardial and the endocardial receptors are connected with afferent fibers of the vagus and depressor nerves; the few myocardial receptors arise from the spinal fibers which approach the heart by way of the stellate ganglion [6, 8, 9]. H. Shaefer [26] states that under ordinary conditions the number of afferent impulses travelling in the cardiac nerves is 20 times the number of efferent impulses. The reflexes from the cardiac interoceptors to the cardiovascular system, and especially in the case of the receptors concentrated in the visceral pericardium, have not been sufficiently carefully investigated. In the majority of the investigations concerned with this question [1, 10] attention is directed mainly toward the pericardial mechanoreceptors; however, the undoubted barrier function of the pericardial sac [11] and the close proximity of the epicardial receptors with a powerful lymphatic network [7] which collects lymph from the whole of the myocardium, poor in receptors, suggest that it is the epicardial chemoreceptors which must be of great importance.

The problem dealt with in the present investigation included the most careful possible study of the character of reflexes associated with different intensities of chemical stimulation of pericardial interoceptors and involving the cardiovascular system, to discover whether these characteristics could be related to the high concentration of sensory endings found in the epicardium. Taking into account that V. N. Chernigovskii studied the effect of chemical stimulation of pericardial receptors on unanesthetized rabbits (whereas the usual experimental animal for chemoreceptor studies in other areas are anesthetized cats) we performed experiments on unanesthetized rabbits, anesthetized rabbits (urethan, hexenal) and anesthetized cats (urethan).

EXPERIMENTAL METHOD

The preliminary operation making it possible to apply stimuli to the pericardial receptors was carried out on rabbits by the V. N. Chernigovskii method [14, 15] and on cats by the C. Drinker method [21]. The aim of both operations was to secure the edges of an opening made in the parietal pericardium to the superficial tissues of the thorax in such a way as to preserve the possibility of natural respiration. Solutions of nicotine in Ringer-Locke solution ($1 \cdot 10^{-9}$ to $1 \cdot 10^{-3}$) served as stimuli; they were applied, with a syringe, directly to the surface of the epicardium in amounts of 0.4-1 ml. The stimulating agent was washed off with pure Ringer-Locke solution. The intervals between stimulations were 10-30 min. Blood pressure was recorded in the carotid artery simultaneously by a mercury and a membrane manometer; respiration was recorded by means of a Marey's tambour connected to a tracheotomy cannula. A total of 30 experiments was performed on rabbits and 100 experiments on cats.

EXPERIMENTAL RESULTS

Repeating the experiments of V. N. Chernigovskii, we obtained similar results: the threshold concentration of nicotine solution which produced a reaction of one sort or another from the cardiovascular system of rabbits when applied to the pericardium was $1 \cdot 10^{-6}$. When the concentration of nicotine is raised to $1 \cdot 10^{-3}$ the reflex is enhanced but unaltered in character, manifesting itself always by a drop of blood pressure and bradycardia.

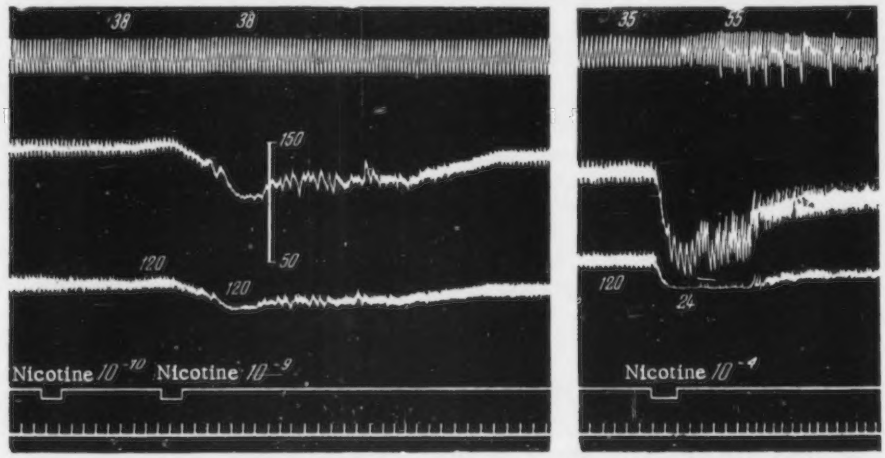

Fig. 1. Circulatory and respiratory reaction of cat to stimulation of pericardial receptors with nicotine in various concentrations. The cat reacted to all concentrations of the stimulating agent only by depressor reactions. Records from above down: respiration, arterial blood pressure (mercury manometer), arterial blood pressure (membrane manometer), stimulus marker, time marker (2 sec). Figures along the tracings recording respiration and blood pressure (membrane manometer) denote respiratory or pulse rate at the given moment calculated per 1 min.

Stimulation of the pericardium with nicotine in cats, unlike unanesthetized rabbits, caused a threshold reflex reaction at considerably lower concentrations of the stimulating agent: $0.4 \cdot 10^{-9}$ to $1 \cdot 10^{-8}$. Moreover, the character of the threshold reaction could be different in different experiments, i. e., pressor or depressor. As the stimulation was increased the sign of the reaction could be either maintained or undergo inversion. Three typical forms of the reaction were demonstrated in different groups of animals. Thus, in one group of animals (15% of the cats), as in rabbits, application of any dose of nicotine (beginning with the threshold) to the pericardium produced a fall of blood pressure and slowing of the heart rate [the cardiac reaction had a somewhat higher threshold (Fig. 1)]; as the stimulation was increased the reaction became greater. In 25% of the animals, application of any concentration of the stimulating agent caused a rise of blood pressure and acceleration of the heart rate (Fig. 2),

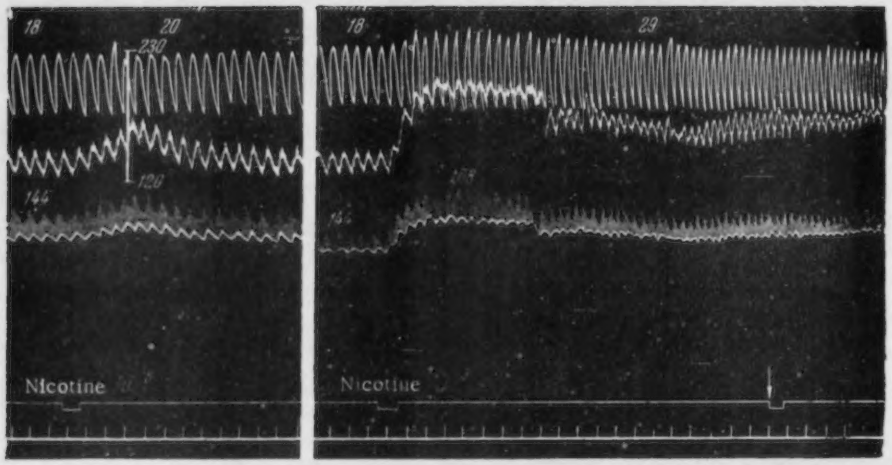

Fig. 2. Circulatory and respiratory reaction in cat to stimulation of pericardial receptors with nicotine in various concentrations. The cat reacted to all concentrations of the stimulating agent only by pressor reactions. Records the same as in Fig. 1. ↓ – washing away of nicotine begun (introduction of 10 ml Ringer-Locke solution into the pericardial cavity).

and increasing the stimulation led to enhancement of both reactions. Finally, most frequently (60% of the cats), increasing the stimulation caused a change not only of the intensity but also of the character of the cardiovascular reaction (Fig. 3): pressor reactions changed to depressor ones. The transition of pressor reactions (elicited in response to weak stimulation) to depressor reactions in response to stronger stimulation was accompanied by replacement of tachycardia by bradycardia; in different experiments this transition occurred when different concentrations of nicotine were used and was sometimes accompanied by the appearance of intermediate diphasic reactions (Fig. 2).

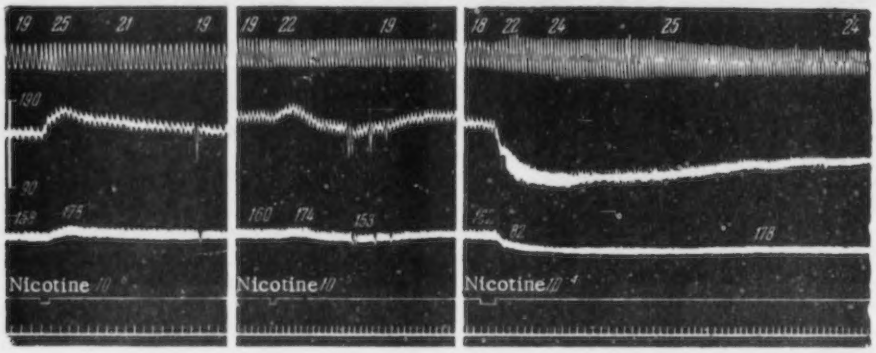

Fig. 3. Transition of the cardiovascular reaction to chemical stimulation of pericardial receptors from pressor to depressor on increasing the concentration of nicotine. Experiment on cat under urethan anesthesia. Records the same as in Fig. 1.

In some experiments, pressor reactions appeared only in response to the weakest stimulation (nicotine $0.4 \cdot 10^{-9}$), larger doses of the stimulating agent causing a fall of blood pressure; in other experiments only the strongest stimulation (nicotine $1 \cdot 10^{-3}$) led to depression, whereas weaker stimuli produced pressor reactions. The boundary between the stimulus strengths eliciting pressor and depressor reactions could lie on any part of the scale of stimulus intensity, but was usually not displaced within the limits of a given experiment.

Respiration always underwent the same change in all the animals irrespective of the direction taken by the cardiovascular reaction: the respiratory movements increased in rate and depth. The threshold of the respiratory reaction was usually higher than the thresholds of the cardiovascular reaction.

Since experiments on unanesthetized rabbits gave results different from those obtained in experiments on cats, we conducted a supplementary series of experiments on rabbits anesthetized with urethan or hexenal. In most of the experiments only depressor reactions were noted, with the exception of 4 out of the 20 experiments.

All the described reactions to chemical stimulation of pericardial receptors are reflex since they disappear after transection of the vagi and depressor nerves in the neck or on introduction of novocain into the pericardial cavity. Data obtained by a number of authors [3, 7, 22, 24, 25] also support the reflex nature of these reactions.

The results obtained in most of the experiments on cats and some on rabbits may be interpreted in two ways: either there are two types of receptors in the pericardium (the excitation of one sort giving rise to tachycardia and rise of blood pressure and excitation of the other to depression and bradycardia) or different degrees of excitation in one or more receptors leads to different results.

W. Douglas and collaborators [19, 20] stimulated the aortic nerve and the vagus in cat, which contain the afferent pathways of the reflexes described by us, and observed 3 different effects: weak stimulation led to a small fall of blood pressure, stronger stimulation resulted in a rise of blood pressure and still stronger stimulation again resulted in a fall of blood pressure, which was more profound than in the former case. The authors explain this result by the presence in the nerves concerned of 3 different types of nerve fibers. However, careful physiologic and morphologic examination carried out by the same authors allows only partial agreement with this: the effect produced by the weakest stimulation of the nerves occurs as the result of stimulation of large fibers associated with the aortic baroreceptors. The authors did not succeed in dividing the fine fibers whose stimulation would cause a rise or profound fall of blood pressure depending on the strength of stimulation. These fibers are evidently associated with the cardiac receptors which were stimulated in our experiments. Consequently, it may be considered that both pressor and depressor reactions elicited by application of nicotine to the pericardium are effected by stimulation of receptors associated with small fibers and that the character of the resultant reaction depends rather on the intensity of stimulation of homogeneous afferent elements than on a hypothetical pressor or depressor specificity of receptors or nerve fibers [4]. Otherwise it would be necessary to suppose that in some animals only "depressor" receptors are present, in others only "pressor" ones, and in the third group of animals both types of receptors, but sometimes one type ceases to manifest itself in the course of an experiment.

M. G. Udel'nov [13] has shown on extensive material that involvement in activity of a small number of efferent elements of the vagus leads to acceleration of the heart rate, whereas increasing the number of simultaneously stimulated homogeneous elements of the vagus produces slowing of the heart rate. This was confirmed by us in experiments on fish [5]. It is possible that the same phenomenon takes place in the experiments described in this work. In our view the transition of pressor reactions to depressor ones on stimulation of the pericardial receptors may be explained by the fact that very large numbers of receptors are present in the epicardium; as the result of this, application of considerable concentrations of the stimulating agent may cause a sufficient flow of impulses to create inhibition in the vasomotor center upon stimulation of this reflexogenic zone.

SUMMARY

Experiments were performed on cats under urethan anesthesia and on cats with normal respiration.

Pericardial receptors stimulated by nicotine ($0.4 \cdot 10^{-9}$ to $1 \cdot 10^{-3}$) cause pronounced reflex changes in the level of the blood pressure, heart rate and respiration. In 60% of experiments, stimulation of pericardial receptors by weak concentrations of nicotine caused increase of the blood pressure and tachycardia, while stimulation by strong concentrations of nicotine resulted in reflex decrease of the blood pressure and bradycardia. In 25% of the experiments, the animals reacted by pressor reactions and tachycardia when pericardial receptors were stimulated by any concentration of nicotine. In 15% of the experiments, any stimulation of these receptors by nicotine caused depression of the cardiovascular system. In all experiments, any chemical stimulation of pericardial receptors both by weak and strong solutions caused increase of the rate and depth of respiratory movements.

It is suggested that the change of the pressor reaction into depressor is caused not by the stimulation of different types of receptors, but depends on the more intense stimulation of a large number of similar receptors.

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CHANGES IN MYOCARDIAL TROPHICS DURING THE CHRONOTROPIC EFFECT

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We showed in a previous communication [1] that under the influence of the dynamic cardiac nerves a change occurred in a number of biochemical activities of the myocardium: tissue respiration, content of glycogen, adenosinetriphosphate (ATP) and creatine phosphate (CP). These changes evidently result from rearrangement of biochemical processes under the influence of the trophic action of the dynamic cardiac nerves.

The present work is concerned with the study of the effect of "rhythmic" nerves which elicit the chronotropic effect, i. e., changes in cardiac rhythm, on the state of the same biochemical factors (tissue respiration, ATP, CP, inorganic phosphorus (IP), glycogen).

Investigations carried out by A. V. Kibiakov and collaborators [2] showed that the "dynamic" and "rhythmic" nerves supplying the heart, whose anatomic entity was proved by I. P. Pavlov, differed also in the mechanism of their action upon the heart. Thus, the inotropic effect of the dynamic nerves is mediated by the essential participation of chemical agents – sympathin and acetylcholine; the rhythmic nerves, however, do not change their influence on the heart even on sharply decreasing the concentration of chemical mediators. It naturally appeared interesting to compare the effect on myocardial trophics of the rhythmic and dynamic nerves – these anatomically independent nervous units whose effect on the object innervated is evidently mediated by different factors.

EXPERIMENTAL METHOD

The experimental method has been described in a previous report. Dogs were used as experimental animals. A total of 56 experiments were performed: 32 with stimulation of the nerves and 24 controls.

EXPERIMENTAL RESULTS

The data obtained by biochemical investigations of the myocardium during positive chronotropic effect of the "rhythmic" sympathetic nerves are summarized in the diagram shown in Fig. 1.

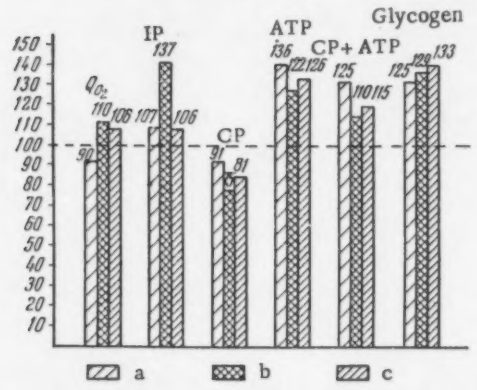

Fig. 1. Changes in the biochemical indices of the myocardium during prolonged chronotropic effect. a) 1st min of stimulation; b) 2nd min of stimulation; 3) 3rd min of stimulation. Figures are given in percentages.

The diagram is based on the arithmetic mean data calculated as percentage of controls. It shows that tissue respiration underwent a diphasic change: initial fall (1st min), followed by a slight rise. The ATP content remained higher than in the control experiments; resynthesis of ATP evidently occurred at the expense of CP whose content remained below the control value throughout the stimulation. According to Chang's findings [3] the mechanical activity of the heart depends mostly on the CP content, and this was confirmed by our experiments.

The amount of glycogen during positive chronotropic effect was increased compared to the control and remained so without significant fluctuations. The dynamics of the changes in tissue respiration and CP content during positive chronotropic effect on the heart led us to the suggestion that acceleration was accompanied by less economic cardiac contraction with the

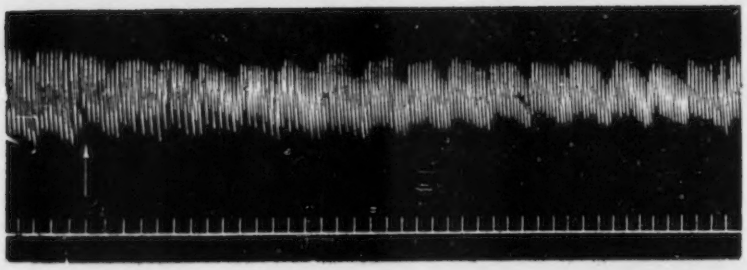

Fig. 2. Effect of stimulation of accelerator nerve (↑) on the character of cardiac activity. Records (from above down): cardiac contractions, time marker (1 sec).

loss of high-energy bonds not fully restored. This may be the very reason for the cardiac exhaustion observed on prolonged action of accelerating nerves. I. P. Pavlov [4] showed that purely accelerator branches, when excited, "diminished the blood flow and the work of the heart."

Figure 2 shows the cardiogram taken from one of the experiments in which stimulation of the accelerator nerve, as well as the corresponding biochemical tests (experiment No. 70, January 5, 1954, see Fig. 1) were carried out. As can be seen from Fig. 2, stimulation of the nerve led to acceleration of the cardiac contractions without an increase in their amplitude, but rather with a slight decrease of the latter, which evidently indicates that purely accelerator nerves were stimulated without admixture of enhancing fibers. Marked reduction of respiration (by 54%) was noted during this, as well as decrease of the CP content by 89%, increase of IP by 127.5% and ATP by 147.9%; the glycogen content also rose by 159%.

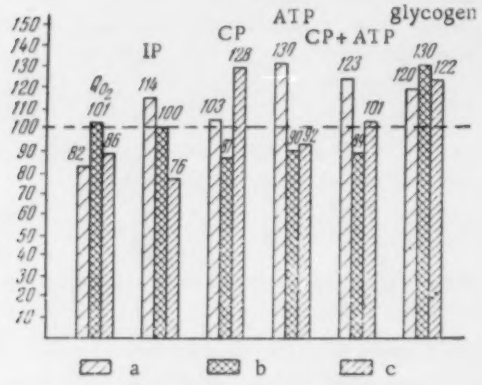

Fig. 3. Change in biochemical indices of the myocardium during negative chronotropic effect. a) 1st min of stimulation; b) 3rd min of stimulation; c) 4th min of stimulation.

The biochemical changes noted during negative chronotropic effect of the "rhythmic" parasympathetic nerves were somewhat different in character from those described: respiration was depressed; the CP content decreased during the 3rd min of stimulation and increased during the 4th; the amount of ATP rose during the 1st min and decreased during further stimulation of decelerator nerve; the IP content, slightly increased during the 1st min of stimulation, gradually decreased; the amount of glycogen rose. The described biochemical changes in the myocardium during a negative chronotropic effect are illustrated in a diagram (Fig. 3); a cardiogram is also presented (Fig. 4).

As can be seen from Fig. 4, stimulation of the nerve led to slowing the rate of cardiac contractions without diminishing their strength. This was associated with an increase in the amount of glycogen by 192%, ATP by 129%, CP by 125% and a slight reduction of IP to 91%.

On the basis of the data obtained, it is thus possible to suggest that the character of the biochemical changes in the myocardium which occur on activation of the rhythm-controlling nerves differs from those changes which are observed during the inotropic effect of the dynamic nerves. Evidently the rhythmic nerves (in contrast to the dynamic nerves) do not produce profound changes in the myocardium which are associated with reorganization of those processes which ensure the energy supply to the heart muscle. During positive chronotropic effect the resynthesis of ATP utilized during contraction of the heart muscle occurs chiefly at the expense of CP which acts as reserve source of high-energy bonds for both the skeletal and cardiac musculature; the mechanical activity of the heart therefore depends on the content of CP.

During negative chronotropic effect the biochemical changes are not great; expenditures incurred during systole are restored during the period of rest or even more than compensated as the result of prolongation of the period of rest between the contractions of the heart.

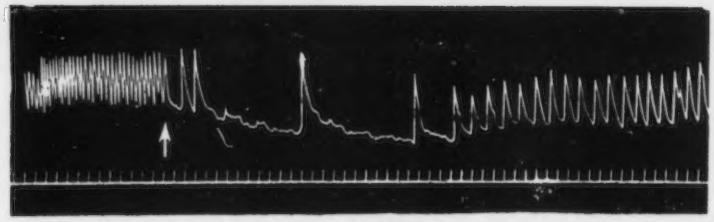

Fig. 4. Effect of stimulation of decelerator nerve on the character of cardiac activity. Records from above down: cardiac contractions, time marker (1 sec). ↑ – time of stimulation.

It is suggested that the rhythmic nerves lead only to certain quantitative changes in the biochemical indices which result from changes in the conditions of cardiac function and not special and profound biochemical reorganization.

SUMMARY

An experimental study was carried out on the effect of rhythmic nerves (causing chronotropic effect) on the change of the cardiac rhythm and tissue respiration, the content of ATP, CP, inorganic phosphorus and glycogen. In positive chronotropic effect, resynthesis of ATP expended on contraction of the cardiac muscle takes place mainly by utilization of CP which is a reserve source of high-energy bonds both for the skeletal and the cardiac muscles. Therefore, the mechanical function of the heart depends on CP content.

In negative chronotropic effect the biochemical changes in the myocardium are insignificant.

In the authors' opinion the rhythmic nerves bring about certain quantitative changes of the biochemical indicaters which are the result of changes in conditions of cardiac function and not special and profound biochemical reorganization.

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THE ROLE OF ACETYLCHOLINE IN THE DEVELOPMENT OF TETANIC SINGLE RESPONSE AND AFTERPOTENTIALS IN THE NERVE TRUNK OF COLD-BLOODED ANIMALS

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The phenomenon of tetanic single contraction described by N. E. Vvendenskii in 1886 [4] is of great theoretical importance since it is a peripheral model of the processes of summation of excitation which occur in the central nervous system. The phenomenon of tetanic single contraction consists of a single wave of excitation, passing through an area of sub-threshold tetany, giving rise to muscle contraction which acquires tetanic character. Academician A. A. Ukhtomskii [14] saw in the mechanism of the formation of a tetanic single contraction such relationships as could take place in the formation of a dominant. A number of authors [1, 10, 11] have shown that the passing wave of excitation activates local processes in the nerve trunk elicited by sub-threshold tetanization. When recording action potentials from the nerve trunk or fiber it is possible to speak not of tetanic single contraction but tetanic single response [11]. A depressed functional state of the nerve trunk forms a favorable condition for the formation of the tetanic single response [4, 10, 13].

The cause underlying the appearance of tetanic single response is considered to be the low voltage negative afterpotential, especially since a connection has been established between the latter and the phase of increased excitability [3, 6, 10, 13, 14, 16]. Many investigators consider that metabolic processes underly the afterpotentials [5, 15]. All the factors which enhance the negative afterpotential lead to improved conditions for demonstration of tetanic single response, and vice versa [3, 13, 14, 17].

The present work is concerned with investigation of the role played by the mediator acetylcholine in the development of negative afterpotential and the phenomenon of tetanic single response, since this aspect has so far received little attention [2, 8].

EXPERIMENTAL METHOD

Experiments were performed on a preparation of n. ischiadicus-n. peroneus of frog (Rana ridibunda). The nerve trunk was placed on 3 pairs of electrodes: maximal stimulation was applied at the proximal electrodes, sub-threshold or threshold tetanization was effected at the middle electrodes, and the distal electrodes were chlorided and used for recording. A grounded silver plate was placed between the middle and distal pairs of electrodes in order to isolate the path of stimulating current. During the experiment the nerve trunk was placed into a specially constructed humid chamber. Two electronic stimulators were used as stimuli. The action potentials were recorded on a two-channel electron oscillograph. Since very low frequencies could be reproduced, it was possible to record almost without distortion the slow afterpotentials in the nerve trunk. During investigation of afterpotentials only 2 pairs of electrodes were used, stimulating and recording. The latter had an interelectrode distance of 2 cm. In order to avoid dispersion in time of the excitation waves in the nerve trunk the distance between the two pairs of electrodes was under 1 cm. To obtain monophasic action-potential waves the peripheral end of the nerve was crushed or treated by heat. The role of acetylcholine was studied by removal of the pancreas, a procedure which, according to a number of investigations [7, 9, 12] impairs the formation of acetylcholine in the organism. The operation for removal of the pancreas was carried out under sterile conditions 4-10 days prior to the experiment. The effect of acetylcholine and eserine was studied by introducing these agents in various concentrations into the posterior lymph sac of frogs. The experiments were performed on winter-spring frogs. A total of 167 experiments was carried out.

EXPERIMENTAL RESULTS

It was possible to obtain tetanic single response fairly frequently at the very beginning of experiment on the nerve trunks of normal frogs when the frequency of sub-threshold stimulation was 50 per second. Such tetanic single response consists of a whole series of action potentials following the frequency of the sub-threshold stimulation quite synchronously and appearing after the action potential elicited by a single stimulus of maximal strength. The amplitude of the action potentials constituting the tetanic single response decreases gradually until complete disappearance. The total duration of the tetanic single response obtained at the very beginning of an experiment is 0.34 sec* (Fig. 1,a). Tetanic single response was never observed at the beginning of an experiment when the frequency of the sub-threshold or threshold stimulation was 25 per second (Fig. 1,b).

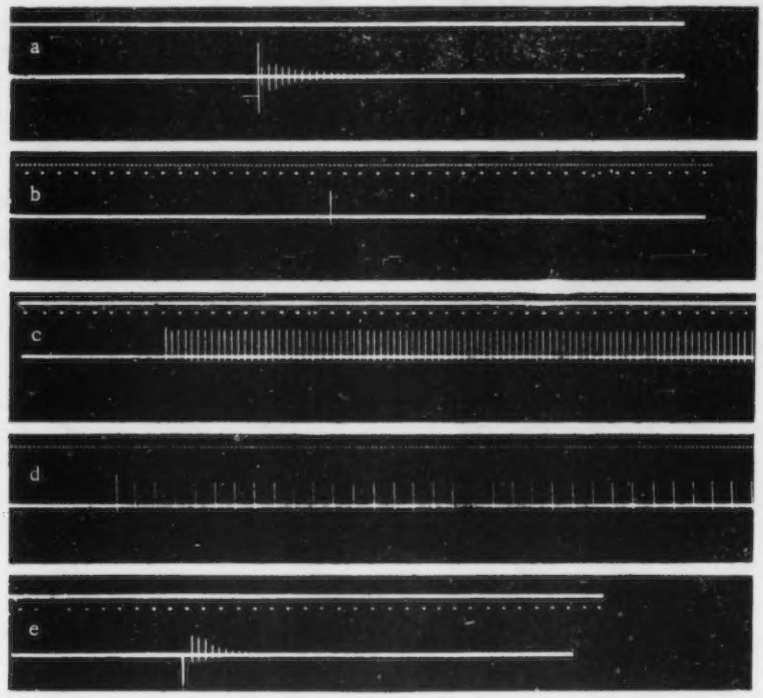

Fig. 1. Electrograms of tetanic single response. a, b) Tetanic single response during 5th min of experiment (frequency of sub-threshold stimulation 50 and 25 per second); c, d) the same on 7th day after removal of pancreas (frequency of stimulation 50 and 25 per second); e) the same on the 7th day after operation with compensatory administration of acetylcholine (frequency of stimulation 50 per second). Records from above down: time marker (0.01 and 0.05 sec), electrogram.

In the case of the nerve trunks of frogs whose pancreas had been removed, tetanic single response could be obtained in all animals without exception on the 6-10th postoperative day; it should be mentioned that from the very first minutes of the experiment the response acquired a "prolonged" character, i. e., the action potentials of the tetanic single response did not show a tendency to disappear rapidly but persisted sometimes up to 30-60 sec with maintenance of quite a high amplitude (Fig. 1,c). Such results were obtained not only with sub-threshold stimulation at a frequency of 50 per second but also at 25 per second (Fig. 1,d). This sort of "prolonged" type of tetanic single response could only be obtained on nerve trunks of normal animals after preliminary supra-threshold stimulation lasting 10-20 min at frequencies of 50-100 per second.

* Average figures are cited in this paper.

Nerve trunks of operated animals who had been systematically given compensatory doses of acetylcholine (1:10,000 solution, 0.5 ml daily from the 3rd-4th postoperative day introduced into the posterior lymph sac) showed tetanic single response at the beginning of experiment which was never "prolonged" (Fig. 1,e); in many cases it was absent altogether.

Introduction of eserine in 1:1000 concentration (1 ml) into the posterior lymph sac of normal animals 40-50 min prior to the experiment caused a sharp deterioration in the conditions for demonstration of tetanic single response; however, eserine in concentration 1:10,000 (1 ml) exerted no significant effect on the course of tetanic single response.

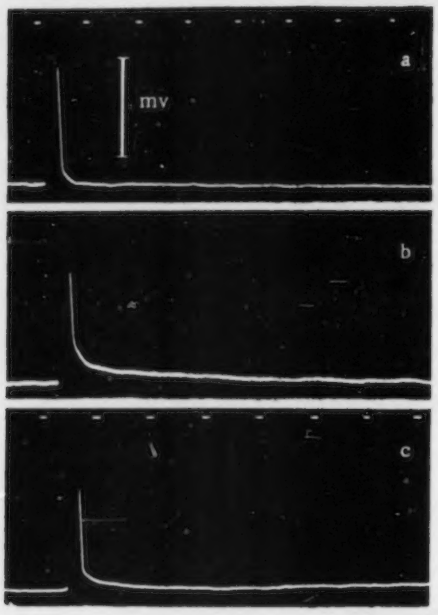

Fig. 2. Electrograms of nerve action potentials. a) Negative afterpotential in nerve of normal frog; b) negative afterpotential on the 7th day after removal of pancreas; c) negative afterpotential of nerve trunk with acetylcholine compensation. The summits of the action potential spikes pass beyond the limits of the records and in all cases reach approximately 4 mv. All the photographs are taken with the same amplification. Calibration 1 mv. Records (from above down): time marker (0.01 sec), electrogram.

Investigation of the negative afterpotential showed that it constituted an average of 4.2% with respect to the spike in the nerve trunks of normal animals; its duration was 0.024 sec (Fig. 2,a). No appreciable positive afterpotential was observed.

Preliminary removal of the pancreas led to marked increase in the negative afterpotential which, under these conditions, constituted 10.27% with respect to the action potential spike and reached 0.1 sec in duration (Fig. 2,b).

Compensatory introduction of acetylcholine to the operated animals from the 3rd-4th postoperative day onwards diminished the negative afterpotential, whose amplitude and duration approached the values obtained from the nerve trunks of normal animals (Fig. 2,c).

Eserine, in concentration 1:1000, administered 40-50 min before the experiment to a normal frog (1 ml) also caused diminution of the negative afterpotential, whose amplitude under these conditions constituted an average of 1.85% with respect to the spike and had a duration of 0.01 sec. Smaller concentrations of eserine (1:10,000, 1 ml) led to slight changes in the negative afterpotential.

Analysis of the experimental results described suggests that the extent to which the negative afterpotential is pronounced depends on the liberation of the mediator acetylcholine.

Studies carried out by A. V. Kibiakov and A. A. Uzbekov have shown that removal of most of the pancreas in frogs causes fatty infiltration of the liver and impairment of acetylcholine synthesis, without significant impairment of sugar metabolism [9]; the impairment of acetylcholine synthesis evidently results from disturbances of fat-lipoid metabolism [12]. According to the data of L. N. Zefirov and A. V. Kibiakov [7], impairment of acetylcholine synthesis leads to lowering of lability, decrease of the resistance of nerve to the action of change-inducing factors.

In our experiments on nerve trunks of depancreatized frogs it was observed that the negative afterpotential was considerably more pronounced, which evidently results from impairment of acetylcholine formation. The presence of quite marked negative afterpotential offers an explanation of the "prolonged" character of the tetanic single response in operated frogs. In this case each wave of excitation of the tetanic single contraction apparently exerts a stronger enhancing effect on the series of following waves which leads to the marked lengthening, "prolongation" in time, of the tetanic single response.

In control experiments in which the operated animals were given acetylcholine the duration of the negative afterpotential decreased and there was concurrent deterioration in conditions with respect to demonstration of tetanic single response.

Our conclusion concerning the significance of acetylcholine in the development of afterpotentials is also confirmed by experiments with eserine. By protecting acetylcholine from rapid hydrolysis, eserine favors diminution of residual processes which, in its turn, determines the difficulty of obtaining tetanic single response in the initial period of an experiment.

The experimental data presented allow the conclusion that acetylcholine favors the diminution of residual processes of excitation in nerve, apparently by exerting a trophic effect.

SUMMARY

Experiments were performed on the nerve trunks of frogs in which the production of acetylcholine was disturbed by removal of the pancreas. This brought about an increase of the negative afterpotential and prolongation of tetanized single response on the 4th to 10th day following the operation. The compensatory administration of acetylcholine removed these changes in the majority of cases. Preliminary administration of eserine to normal frogs resulted in decrease of the negative afterpotential. With administration of eserine the conditions for appearance of the tetanized single response deteriorated. Evidently, acetylcholine promotes the decrease of the duration of residual processes of excitation in the nerve tissue.

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PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

CHANGES IN THE DURATION OF THE VENTRICULAR COMPLEX OF THE ELECTROCARDIOGRAM DURING INTERRUPTION AND RESTORATION OF THE WORK OF THE HEART

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In the opinion of the majority of workers, the duration of the ventricular complex of the electrocardiogram (Q – T interval) reflects the duration of the ventricular contraction of the heart and is an important indication of its activity [9, 11, 14]. In the case of weakening of the contraction of the heart, the duration of the ventricular complex as a rule increases [13, 19, 20, 22]. There are, however, indications of the possibility of shortening of the ventricular complex during apparent death, both clinically [15, 18, 26, 28] and in experiments on the hearts of warm- and cold-blooded animals [3, 7, 21, 24]. Restoration of cardiac activity is accompanied by the converse sign – lengthening of the Q – T interval. Later on however, concurrently with strengthening of the contraction of the heart, the duration of the ventricular complex gradually decreases [1, 3, 7, 21].

In a previous report [3] the hypothesis was proposed that differences in the duration of the ventricular complex during apparent death and resuscitation were due to changes in the number of myocardial cells exhibiting bioelectrical activity.

In the present paper we give the results of further investigation of conditions affecting the duration of the ventricular complex: the influence of the character and duration of the process of apparent death, the duration of clinical death, the type of anesthesia used in the experiment and various other factors.

EXPERIMENTAL METHOD

Experiments were performed on 115 adult dogs weighing from 6 to 25 kg. In the majority of the experiments the animals were given a preliminary subcutaneous injection of pantopon (8 mg per 1 kg body weight) and in about 50% of the experiments general ether anesthesia was used also. Clinical death was brought about in 85 dogs by exsanguination, in 20 dogs by asphyxia and in 10 dogs by electrical trauma. Resuscitation measures began at various intervals of time after the onset of clinical death (from 1 to 16 min). These consisted of artificial respiration and intra-arterial transfusion of blood under pressure with the addition of glucose and adrenalin by a method devised by V. A. Negovskii and his co-workers [4, 5]. In some experiments adrenalin was not given.

The electrocardiograms were recorded on a portable electrocardiograph. The action currents of the heart were tapped by means of needle electrodes inserted into the skin at the level of the upper and lower ends of the body of the sternum.

EXPERIMENTAL RESULTS

Analysis of the electrocardiographic records made during various forms of apparent death showed that changes in the duration of the ventricular complex during clinical death and subsequent resuscitation were determined mainly by the degree of anoxia of the heart, i. e., by the duration of the lethal process and of the state of clinical

Relationship Between the Duration of the Ventricular Complex and the Degree of Anoxia of the Heart

Expt. no.	Cause of death	Duration of		Duration of ventricular complex		Time of restoration of the work of the heart	No. of expts.
		lethal process	clinical death	during clinical death	during resuscitation		
		minutes		seconds			
1	Electrical trauma	2—7	1—3	0.14—0.19	0.23—0.35	25—32	10
2	Asphyxia	6—9	1—4	0.15—0.18	0.25—0.36	24—31	12
3	Exsanguination	4—10	3—5	0.16—0.22	0.27—0.37	22—37	10
4	»	6—12	7—10	0.16—0.27	0.30—0.55	30—48	4
5	»	8—14	15—16	0.26—0.31	0.45	Did not recover	2

death (see table). After a rapid process (less than 10 min) the duration of the ventricular complex was reduced in the first minutes of clinical death to 0.14-0.22 sec (before the experiment the Q – T interval was 0.20-0.27 sec, and after injection of pantopon 0.24-0.30 sec; Figs. 1, 2, 3). Changes in the form of the ventricular complex at this time consisted of the appearance of a deep S wave, and displacement of the S – T interval and its merging with a high T wave. After a more prolonged lethal process (15-30 min) the shortening of the ventricular

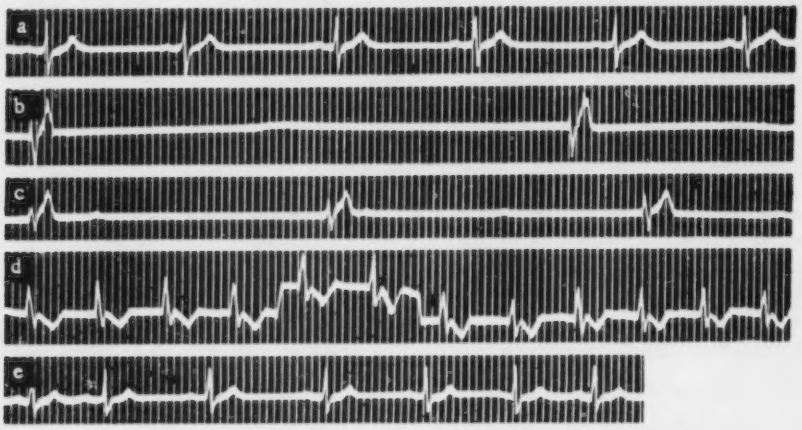

Fig. 1. Changes in the electrocardiogram during clinical death lasting 5 min (caused by hemorrhage lasting 8 min). a) Before hemorrhage Q – T was 0.26-0.27 sec; b) 3 min of clinical death: diminution of Q – T to 0.17-0.18 sec; c) beginning of resuscitation: lengthening of Q – T to 0.19-0.22 sec; d) 30 sec from the beginning of resuscitation: appearance of effective contractions of the heart, lengthening of Q – T to 0.28-0.30 sec; e) 20 min from the beginning of resuscitation: Q – T equal to 0.22-0.24 sec.

complex during clinical death was less pronounced or even could not be found (see Figs. 1,b-3,b). The form of the ventricular complex was changed considerably more in these experiments: often total merging of the initial and terminal parts of the complex was observed with the formation of a typical monophasic deflection (see Fig. 3,c).

In the later stages of clinical death (7-10 min) the duration of the ventricular complex was increased. Subsequently, a secondary reduction in the duration of the complex could be seen with an alteration in its shape and diminution of its amplitude (see Fig. 3, b, d, f). A similar feature – increase in the duration of the ventricular complex and its subsequent shortening with increasing anoxia – was observed also in the case of secondary interruption of the work of the heart following its temporary restoration (see Fig. 3, i).

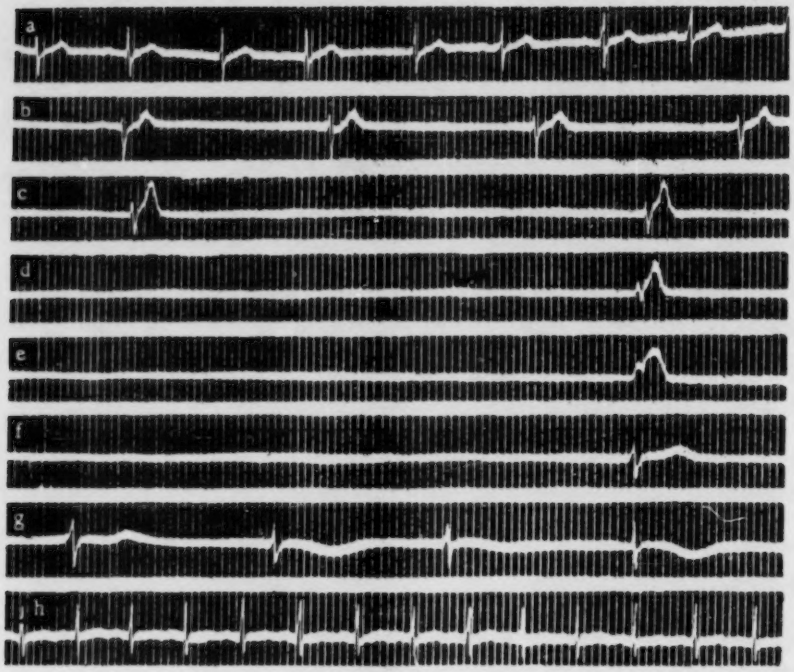

Fig. 2. Changes in the electrocardiogram during clinical death lasting 10 min (caused by hemorrhage lasting 7 min). a, b) Before hemorrhage and 4 min after its start: duration of Q – T 0.20 and 0.22 sec, respectively; c, d, e) 3rd, 5th and 8th min of clinical death: duration of ventricular complex in the region of 0.2 sec; f) beginning of resuscitation: lengthening of Q – T to 0.42 sec; g) 50 sec from the beginning of resuscitation: appearance of effective contractions of the heart, lengthening of Q – T to 0.54 sec; h) 28 min from the beginning of resuscitation: Q – T equal to 0.20 sec.

During the performance of resuscitation measures, simultaneously with restoration of the form of the ventricular complex, its duration increased. The degree of this increase was variable, depending on the duration of the anoxia. After rapid production of apparent death (less than 10 min) and a short period of clinical death (less than 5 min) the duration of the ventricular complex rose to 0.23-0.37 sec. On restoration of the cardiac activity after a more lengthy lethal process (10-15 min) or a more prolonged period of clinical death (7-10 min) the duration of the complex increased to 0.3-0.55 sec (see table and Figs. 1 and 2). The absence of a rapid and significant lengthening of the ventricular complex during resuscitation was a bad prognostic sign. This feature was usually observed after prolonged periods of agony and clinical death, when the cardiac activity was restored extremely slowly or not at all (see table and Fig. 3, g and h).

No connection was established between the changes in the duration of the ventricular complex during clinical death and the subsequent restoration of cardiac activity and the frequency of the rhythm.

Slowing of the electrocardiographic complexes after the onset of clinical death was accompanied in most cases by shortening of their duration; increase in the rate during resuscitation, on the other hand, was accompanied by lengthening of the complexes. The absence of any relationship between the duration of the ventricular complex and the rate of the rhythm is shown clearly by comparing the electrocardiograms recorded during production of apparent death and resuscitation: with the same rate of the rhythm the duration of the Q – T interval is considerably longer in resuscitation than in production of apparent death (see Fig. 3, b, g and Fig. 2, b, g).

The method of anesthesia used during the experiments did not have any essential influence on the changes in the action currents of the heart during clinical death and subsequent resuscitation. Only with deep and prolonged ether anesthesia were more persistent anoxic changes produced in the electrocardiogram, which were superimposed upon the changes arising during agony and clinical death and enhanced the corresponding disturbances of form and duration of the ventricular complex.

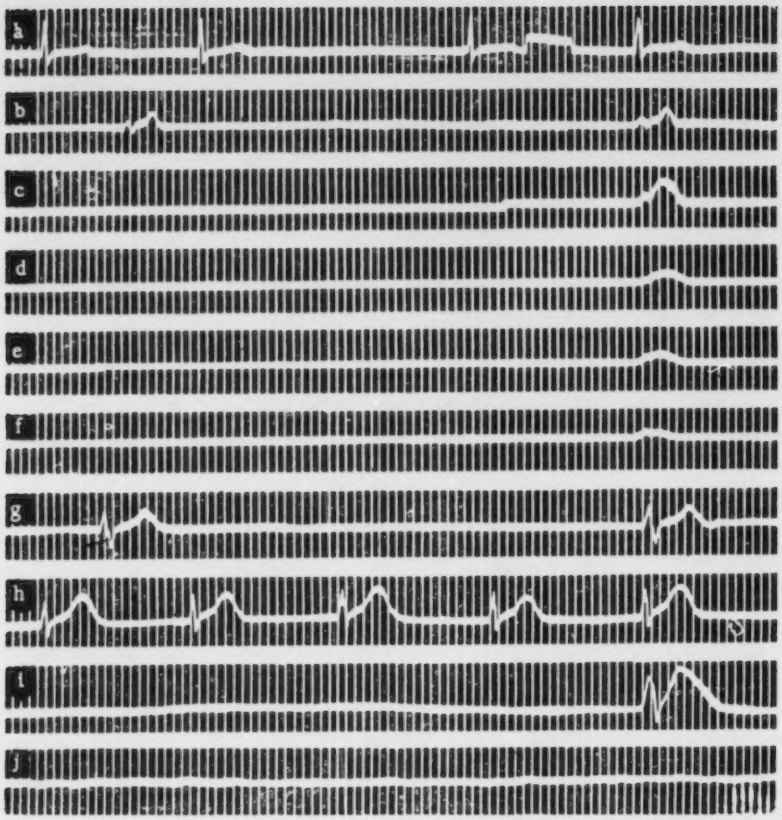

Fig. 3. Changes in the electrocardiogram during clinical death lasting 16 min (caused by hemorrhage lasting 14 min). a) Before hemorrhage Q – T was 0.3 sec; b, c, d, e, f) 3rd, 5th, 7th, 9th and 12th min of clinical death; duration of ventricular complex 0.23, 0.25, 0.30, 0.27 and 0.20 sec, respectively; g) 1.5 min from the beginning of resuscitation; lengthening of Q – T to 0.37-0.40 sec; h) 9th min of resuscitation; temporary restoration of weak cardiac activity, Q – T 0.35-0.40 sec; i) secondary interruption of the work of the heart; j) appearance of ventricular fibrillation.

On infusion of blood under pressure without the addition of adrenalin the cardiac activity was restored more slowly or not at all. In these experiments the changes in the electrocardiogram were produced more slowly and the Q – T interval was lengthened to a lesser degree. This shows that the injection of adrenalin at the time of resuscitation brings about restoration of the form of the ventricular complex, lengthening of its duration and the more rapid restoration to normal of the effective work of the heart.

In view of reports in the literature on the relationship between the Q – T interval and the filling of the heart with blood [10, 19, 23, 25], it might be thought that reduction in the duration of the ventricular complex during production of apparent death from exsanguination was due to reduction in the volume of the heart. In this case, lengthening of the Q – T interval during resuscitation might be explained by increase in the ventricular volume as a result of the infusion of blood. However, the analogous reduction in the duration of the ventricular complex observed during production of apparent death by asphyxia or electrical trauma, in spite of the considerably greater volume of the ventricles, does not support such a hypothesis.

These changes in the duration of the ventricular complex which have been described are most likely to be connected with the qualitatively distinguishable changes in conductivity during production of apparent death and

resuscitation. The reduction in the duration of the ventricular complex during clinical death can be explained, in the view of N. L. Gurvich [2], by the smaller number of myocardial fibers taking part in the excitation, in consequence of the development of a block to the conduction of the excitation. This hypothesis is confirmed by the changes in the form of the ventricular complex observed at this time: the splitting up and widening of its initial part (QRS); displacement of the S – T interval and the formation of a monophasic deflection (see Figs. 1, 2, 3). According to the generally accepted idea, such changes in the electrocardiogram result from disturbance of intraventricular conductivity and the exclusion of part of the myocardium from the process of excitation [8, 10, 11, 30].

The relatively long duration of the ventricular complex after prolonged agony and clinical death and secondary interruption of the work of the heart may be explained by the slowing of conduction which coincides with the increasing anoxia. This slowing of conduction masks the reduction in the number of myocardial fibers taking part in the excitation. With a longer period of clinical death, the secondary shortening of the ventricular complex is evidently due to further disturbance of conduction and the still greater reduction in the number of myocardial cells showing bioelectrical activity.

In accordance with the above, the increased duration of the ventricular complex during restoration of the work of the heart may be explained by removal of the block of intraventricular conduction present during clinical death and by increase in the number of myocardial cells taking part in the excitation. The extremely long duration of the ventricular complex at this time (up to 0.3-0.4 sec in place of the normal 0.2-0.27 sec) can be attributed to the retardation of the spread of excitation through the myocardium which is natural in such a condition. This hypothesis agrees well with the even greater increase in the duration of the ventricular complex (up to 0.55 sec) after a more prolonged production of apparent death and a more prolonged period of clinical death.

This also explains the regular coincidence of changes in the electrocardiogram and the rate of restoration of the effective work of the heart. Where there is a rapid change in the electrocardiogram and pronounced increase in the duration of the ventricular complex, the effective work of the heart is restored rapidly. With slow restoration of the form of the ventricular complex and slow and slight increase in its duration, the restoration of cardiac activity is also observed to be slow. The connection between the alteration in the form of the ventricular complex and its duration with the effective work of the heart is evidently due to the fact that each of these signs depends on the rate of restoration of intraventricular conductivity.

Similar findings were obtained by Bauereisen [12]. By artificial stimulation of the frog's heart with an excessive supply of oxygen this worker observed a diminution in the amplitude and duration of the mechanogram of the heart with increasing exhaustion. In oxygen lack the duration of the contraction was only slightly affected, but the amplitude was reduced as before. It has to be pointed out that these changes in the duration of the contraction of the heart are to some extent analogous to those which we observed in the duration of electrical systole depending on the degree of anoxia.

Weakening of the contraction of the heart by vagal inhibition is also known to be accompanied by reduction in the duration of the ventricular complex [6, 16, 17, 29]. It is very probable that in this case the interference with conduction takes place up to and including total blockade of the terminal branches of the conducting system, thereby isolating the myocardial fibers from the process of excitation. This idea of the possibility of partial asystole during vagal inhibition was expressed by Rothberger and Winterberg [27], who regarded this phenomenon as analogous with the weakening of the contraction in pulsus alternans.

It can be concluded from these findings that the changes in the duration of the ventricular complex during apparent death and resuscitation are determined by the degree of interference with intraventricular conduction. Its slowing after the prolonged production of apparent death causes increase in the duration of the ventricular complex. The extreme degree of disturbance of conductivity arising during clinical death leads to blockade of the terminal branches of the conducting system and to reduction in the duration of the ventricular complex. The combination of these two degrees of disturbance of conduction determines the duration of the ventricular complex during clinical death. Changes in the duration of the ventricular complex during resuscitation depend on the degree of restoration of intraventricular conductivity, more particularly on the number of myocardial fibers taking part in the excitation and on the rate of spread of the excitation throughout the ventricle.

SUMMARY

ECG changes were studied in the agonal condition and during resuscitation of dogs. After quick death the ventricular complex usually shortened to 0.15-0.20 sec during the first minutes of the clinical death, while in re-establishment of the heart work it was increased to 0.25-0.35 sec. In a prolonged agony and clinical death the ventricular complex was not shortened as much, while in resuscitation it was prolonged to 0.4-0.55 sec.

Various changes which take place in the length of the ventricular complex in the processes of dying and resuscitation may be explained by the relationship of two factors: the velocity of the spread of excitation and the number of the myocardial elements which are excited.

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THE EFFECT OF LONG-STANDING EXPERIMENTAL ARTERIOVENOUS ANEURYSMS ON THE HEART

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The influence of arteriovenous aneurysms on the central and peripheral circulation has been the subject of a considerable number of monographs and papers. This fact demonstrates the unfailing interest of workers in various countries in this field of pathology. There are several experimental investigations [4-8 and others] which supplement the findings of clinical observations. However, certain features of the complex pathogenesis of the cardiovascular disturbances arising with arteriovenous aneurysms still remain unexplained; in particular the mechanism of development of bacterial endocarditis and endarteritis in the fistula. In the world literature only a few papers have been published on the experimental production of endocarditis in these conditions [10-12].

In the present investigation we set out to study the effect of arteriovenous fistulae on the heart in experiments of long duration (up to one-and-a-half years) using a combined method of investigation. This consisted of radiography of the heart, electrocardiography, determination of the rate of the cardiac rhythm, and pathological-anatomical and histological study of animals dying or killed at different times; other investigations included temperature recording, blood cultures and measurement of the ESR. This method (combined investigation) enables the progress of the changes in the heart of the experimental animals to be observed more fully and comprehensively.

EXPERIMENTAL METHOD

The experiments were performed on 20 dogs weighing 15-20 kg. Arteriovenous fistulae were produced aseptically in the femoral vessels; in 4 animals an anastomosis was produced between the abdominal aorta and the inferior vena cava. The femoral fistulae were produced as a rule in both limbs with an interval of from 2 weeks to 2 months between the operations.

In order to expose the femoral vessels the superficial tissues were divided along the line of the femoral artery. Access to the abdominal aorta and inferior vena cava was secured by a midline incision in the anterior abdominal wall. The loops of bowel were partly displaced upwards and partly eventrated onto a sterile towel thoroughly soaked in warm physiological saline. Linear incisions along the course of the arterial and venous trunks were made easily by the use of an instrument specially made for the purpose – a "vascular probe." This consists of an elongated cone with a pointed apex, on the surface of which a narrow longitudinal groove is made. With a continuous suture the medial border of the incisions in the artery and vein is first sutured, and then the lateral. Altogether 35 functioning arteriovenous fistulae, 1-3 cm in diameter, were produced.

X-rays of the chest were taken in the frontal plane with a portable RU-760 x-ray apparatus, with the heart in the same position relative to the tube of the apparatus. Films were taken before operation, immediately after operation, and then regularly every 10-20 days.

The electrocardiograms were always recorded under the same conditions, in the standing position (in a frame). The action currents of the heart were recorded by 3 standard leads from the limbs by an EKP-4 apparatus with a constant sensitivity of 1 mv = 10 mm. The initial electrocardiographic baseline was established after the total extinction of reflexes to the environment [9]. After the fistula was created, the first electrocardiogram was taken not immediately but after a few days. This avoided abnormalities caused by incidental factors (anesthesia, pain, changes in the position of the body and so on). Subsequent recordings were made periodically, at the same intervals as the x-rays. In the whole combined investigation about 150 x-rays and over 100 electrocardiograms were taken, processed and analyzed.

EXPERIMENTAL RESULTS

At any time of the existence of arteriovenous aneurysms (Fig. 1) the characteristic sound could be heard on auscultation on the site of a fistula – continuous, low pitched, with intensification and raising of the tone during systole; a sensation of fibrillation on palpation corresponded to the bruit. In the femoral anastomoses we observed the rapid development of venous collaterals.

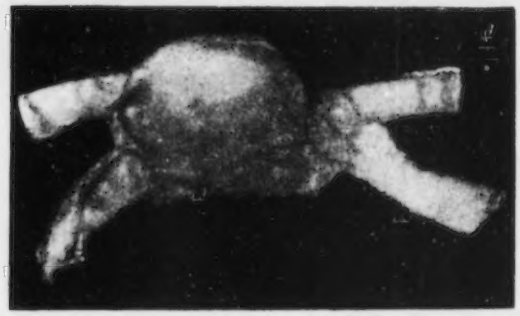

Fig. 1. Experimental arteriovenous aneurysm of the femoral vessels.

Relationship of the Pulse Rate to the Size of an Experimental Arteriovenous Fistula of the Femoral Vessels

Size of fistula in cm	Pulse before creation of fistula	Pulse after creation of fistula	Degree of pulse quickening (beats/min)
1.5	84	102	18
2.0	95	118	23
2 5	107	140	33

In all the experimental animals, changes were observed in the cardiac rhythm, which was increased in rate. With the formation of a second arteriovenous fistula in the same animal, a new rise in the rate of cardiac contractions took place. On compression of the fistula or of the artery leading to it the pulse was slowed (the so-called Dobrovol'skaia's sign). The degree of quickening of the rhythm of cardiac activity increased with increasing diameter of the fistula (see table) and its nearness to the heart. These features observed have a reflex mechanism [1] and are characteristic of the adaptative reaction of the cardiovascular system to the altered conditions of the circulation.

The x-rays obtained showed increase in the heart dimensions, usually developing gradually over a period of several months. Immediately after the formation of the aneurysm it was not possible to find any difference from the original condition (silhouette). The development of this feature was seen more clearly in animals with double femoral arteriovenous fistulae of large size, although the enlargement was observed also in the case of a single femoral fistula (Fig. 2, a, b). In every animal we observed changes in the contour of the heart up to a certain limit. The degree of enlargement, however, was directly proportional to the load on the heart resulting from the development of the pathological anastomoses.

Ligation of the fistula did not cause any noticeable alteration in the size of the heart, which suggested hypertrophy of the myocardium, subsequently confirmed macro- and microscopically. With the development of hypertrophy of the cardiac muscle the pulse always became rather slower. This can be regarded as a favorable prognostic sign, indicating high reserves of power of the heart. In certain dogs with long-standing functioning aneurysms (over a year) the slowness of the pulse was so pronounced that the difference from the preoperative rate was abolished; Dobrovol'skaia's sign was however always present in these cases.

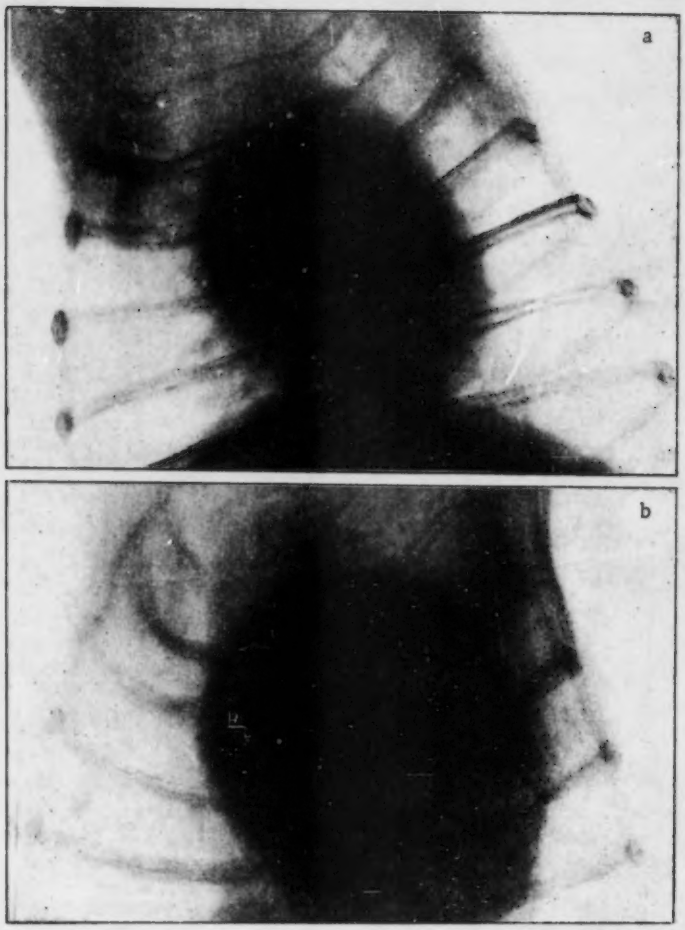

Fig. 2. X-ray of a dog's heart before the creation of an arteriovenous anastomosis (a); x-ray of the heart of the same dog 11 months after operation (b).

By means of electrocardiograms we established considerable changes in the biochemical processes in the cardiac muscle, appearing in even an early stage of the existence of arteriovenous anastomoses, and taking the form of reduction in the voltage and inversion of the T wave. At a later period various animals developed changes which indicated slowing of atrioventricular and intraventricular conduction, and disturbance of excitation in the atria and ventricles. This was shown by lengthening of the P – Q interval, widening of the QRS complex, enlargement and widening, and sometimes smoothing out of the P wave, voltage changes in the R wave, displacement of the S – T interval and deepening of the Q wave. The duration of electrical systole was increased. The direction of the electrical axis of the heart was altered. Investigations over a period of time showed a definite relationship between the magnitude of the changes and the duration of the fistulae, their position and size. Our electrocardiographic results are in agreement with the findings of B. V. Ognev [5].

Comparison of the x-ray and electrocardiographic signs with the results of post mortem examination showed their morphological basis. In animals with profound changes in the elements of the electrocardiogram there were well-marked pathological changes in the heart. The cardiac muscle appeared flabby; the heart was increased in weight, and the walls of both ventricles thickened in comparison with those of animals identical in weight and growth with the experimental ones. Hypertrophy of the left ventricle usually predominated, which corresponded to the rotation of the electrical axis of the heart on the electrocardiograms. Histological examination of the myocardium revealed thickening of the muscle fibers, foci of hemorrhage, edema of the interstitial tissue and cellular infiltrations mainly perivascular in distribution – the appearance of focal and diffuse myocarditis.

In the majority of the dogs valvular abnormalities were found, which were characteristic of bacterial endocarditis; thrombotic deposits on the surface of the folds, areas of fibrinoid necrosis, collections of bacteria and leucocytic infiltration with subsequent increase in the number of lymphoid and histiocytic cells. Lesions of the mitral valve were most common. Less commonly and to a lesser degree the tricuspid and aortic valves were involved in the process. In the heart of these dogs during life, systolic and diastolic murmurs could be auscultated, the character and intensity of which varied. In some animals with long-standing aneurysms, focal nephritis was present; in one case an extensive infarct of recent origin was discovered in the kidney.

In the region of the arteriovenous fistulae a well-defined inflammatory reaction was present, with thickening of the intima, fibrinous deposits, foci of necrosis and cellular infiltration, and a multitude of newly formed vessels. Sometimes endarteritis could be detected at the site of the fistula only, without any visible lesion in the valves of the heart.

In dogs with signs of endarteritis and endocarditis, periodic increases in the temperature were observed, accompanied by increases in the pulse rate and a raised ESR, and blood cultures yielded Streptococcus viridans (by the biological method of N. V. Zhuravskaia [3] and the usual bacteriological method). Blood cultures from the control group of animals were sterile.

The pathogenesis of the cardiac lesions which we observed appears to be as follows. With the formation of an experimental fistula between the artery and vein, part of the arterial blood enters the venous system, leading to an increase in the pressure in the venae cavae and overfilling of the right heart. The animal responds to this sudden change in the conditions of the circulation by a complex reflex mechanism increasing the minute volume by means of quickening of the heart beat. If in each individual case the tachycardia does not exceed the capabilities of the heart (acute cardiac failure often arises), then in the course of time it is compensated to a certain extend by hypertrophy of the myocardium. Since the load on the left heart is greater (to prevent a fall in the peripheral arterial pressure and with overfilling of the right heart), the main hypertrophy affects the left ventricle. The "reorganization" of the cardiovascular system primarily is responsible for the intimate trophic changes in the myocardium, reflected in the changes in the T wave of the electrocardiogram.

The etiology and pathogenesis of bacterial endocarditis and endarteritis are very complex in both man and animals.

Microorganisms constantly enter the blood stream through the mucous membranes, the lungs, the skin and gastrointestinal tract. However, in normal conditions, a developing infection is easily disposed of by the body without the formation of local foci of infection or general septicemia. The arteriovenous fistula and its associated hemodynamic disturbances evidently provide unfavorable conditions for the body to carry out its protective functions. The relatively easy development of bacterial lesions is proved in situations affected by pathological processes. The region of the fistula of an arteriovenous aneurysm, with previous damage to the intima, is most probably a very suitable place for the formation of an inflammatory focus (bacterial endarteritis).

An arteriovenous fistula which is a focus of chronic pathological irritation undoubtedly exerts a neuro-allergic action on the reactivity of the internal membrane of the heart [2]. Furthermore the aneurysm increases the susceptibility of the endocardium (and the myocardium) to infection on account of the overstrain of the cardiovascular apparatus which it causes. Hence on these grounds, and considering the severe strain mainly on the left heart, the more frequent development of the infective process in our experiments on the mitral and aortic valves is accounted for.

The progressive endarteritis of the arteriovenous fistula may play a part as a source of the periodic discharge of bacteria into the blood stream, although it is difficult to discount other portals of infection. Since the aneurysms were created aseptically but blood cultures yielded a growth of Streptococcus viridans, it is most likely that autoinfection takes place.

Our experimental investigations thus confirm the clinical observations of complex changes in the heart arising with congenital and traumatic arteriovenous aneurysms. Bacterial endarteritis develops in the region of the fistula itself. In this way various conditions are created which are favorable to the development of infective endocarditis, even without the deliberate introduction of bacteria. The accompanying bacteremia (Streptococcus viridans) is evidence of the autoinfectious character of the infective process.

The production of infective endocarditis experimentally, by an original method (without previous introduction of microorganisms, damage to the heart valves or sensitization of the animal), approximating the clinical course and pathomorphological and bacteriological features of the chronic bacterial endocarditis of man, opens up wide possibilities for research into this disease in several directions.

SUMMARY

Chronic experiments on dogs with formation of arteriovenous fistulae support clinical observations on the complex changes in the heart which take place in congenital and traumatic arteriovenous aneurysms. Endarteritis appears at the site of the anastomosis. Certain conditions are created favoring the development of septic endocarditis even without the inoculation of bacteria. Associated bacteremia (Streptococcus viridans) permits the assumption that the septic process is autoinfective.

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THE ROLE OF THE HEMATOENCEPHALIC BARRIER IN THE PATHOGENESIS AND THERAPY OF ACUTE CEREBROSPINAL TRAUMA

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Clinical observations show that acute cerebrospinal trauma, like certain diseases of the central nervous system, is accompanied by disturbance of the functional state of the hematoencephalic barrier [1, 2].

In the present investigation* we attempted to clarify the physiological mechanisms bringing about an increase in the permeability of the hematoencephalic barrier in cerebrospinal trauma, and also to seek methods of reducing it. Since in cerebrospinal trauma there is an accumulation of acetylcholine in the tissues of the brain and the cerebrospinal fluid [4, 5, 6] which may cause increased permeability of the hematoencephalic barrier because of increase in the activity of the tissue hyaluronidases [3]; concurrently with the study of the permeability we determined the acetylcholine content and the hyaluronidase activity of the cerebrospinal fluid.

EXPERIMENTAL METHOD

In order to determine the permeability of the hematoencephalic barrier we used radiophosphorus (P^{32}), which was given orally to the subjects of the investigation in a dose of 80-100 mC. From 45-60 min after administration of the P^{32}, lumbar puncture was performed, and then over a period of time repeated examinations of the cerebrospinal fluid and blood were made (in patients in whom lumbar puncture was indicated for diagnostic or therapeutic purposes).

The radioactivity of the test fluids was determined by means of a counter with a type B apparatus. The results were expressed as percentages of the content of P^{32} in the blood.

The acetylcholine was investigated by a biological method in the dorsal muscle of a leech against a standard acetylcholine solution.

Hyaluronidase was estimated by McClean's method of mucin precipitation.

EXPERIMENTAL RESULTS

Our observations were made on 100 patients with acute cerebrospinal trauma. The patients were admitted to the hospital within a few days of the injury.

Two clinical groups were distinguished in accordance with the severity of the trauma and the findings of the permeability of the hematoencephalic barrier: patients with moderately severe and those with severe forms of injury.

We also distinguished a group of patients with slight head injuries without signs of concussion of the brain, and on investigation of the permeability of the hematoencephalic barrier in this case the findings were taken to be normal.

In addition we investigated the distribution of P^{32} in the brain of 8 patients who were given the isotope previously and who died as a result of severe cerebrospinal trauma two days before examination.

The results obtained showed that the permeability of the hematoencephalic barrier in slight injuries without signs of concussion of the brain is 1.5-3%, which evidently represents the normal permeability to P^{32}; in trauma of moderate severity the permeability is raised to 7.5-9%; in severe trauma it amounts to 11-12% and sometimes reaches 25-30%, which agrees completely with the findings of A. M. Vein [1].

* The work was carried out under the guidance of Prof. G. N. Kassil'.

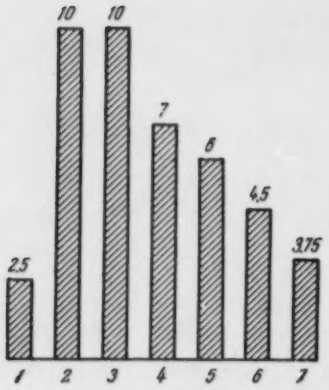

Fig. 1. Content of P^{32} in the cerebrospinal fluid (in percentages of its content in the blood; average data of all the investigations made). 1)Normal; 2) in closed trauma of the skull; 3) in closed trauma of the skull after administration of atropine; 4) in closed trauma of the skull after administration of phenylephrine; 5) in closed trauma of the skull after administration of prostigmin (moderate and severe forms); 6) in closed trauma of the skull after administration of pentamine; 7) in closed trauma of the skull after administration of benadryl.

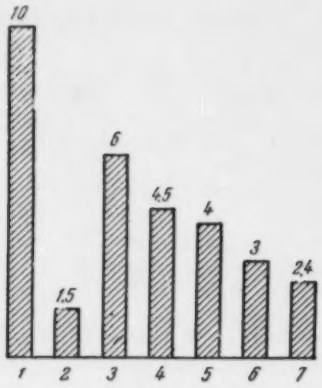

Fig. 2. Content of acetylcholine in the cerebrospinal fluid in γ% (average results of all investigations). 1) Standard solution of acetylcholine – 10%; 2) normal; 3) in closed trauma of the skull; 4) in closed trauma of the skull after administration of atropine; 5) in closed trauma of the skull after administration of phenylephrine; 6) in closed trauma of the skull after administration of prostigmin; 7) in closed trauma of the skull after administration of pentamine.

The acetylcholine concentration in the cerebrospinal fluid does not normally exceed 1-1.5 γ%. In severe head injury its content was raised to 6-7 γ%.

In severe trauma, in addition to increased permeability of the hematoencephalic barrier, increased activity of hyaluronidase in the cerebrospinal fluid was observed.

When the brain of patients dying from the sequelae of trauma was investigated rapid penetration of P^{32} into the brain was found: even 30 min after the administration of P^{32} to the patients it could be found in the brain tissue in considerable quantity.

The intensity of accumulation of P^{32} depends on the period elapsing from the time of its administration until death. P^{32} is distributed in the brain tissue as follows: hypophysis > vascular plexi > diencephalic region > > cerebral cortex > brain stem > white matter.

It was found by examination of symmetrical areas of the brain that in regions in which pinpoint hemorrhages were present without mechanical damage to the vessel walls, there was a considerably larger accumulation of P^{32} in comparison with macroscopically unchanged areas of the brain.

In contused areas of the cerebral cortex the accumulation of P^{32} was considerably greater than in the undamaged cortex or in the cortex where small subpial hemorrhages were present. If the high content of P^{32} in the contused areas is connected with mechanical damage to the walls of the vessels, in the region of the subpial and pinpoint hemorrhages, as shown by control histological investigations, no mechanical damage to the vessel walls could as a rule be detected, and for this reason it must be considered that the high content of P^{32} in these regions is due to a sharp increase in the permeability of the vascular walls, through which may pass not only plasma but also the blood cells.

In view of the functional disturbances established, in the second part of the work we tested the activity of anticholinergic (atropine), adrenergic (phenylephrine), anticholinesterase (prostigmin) antihistamine (benadryl) and ganglion-blocking (pentamine) drugs on the altered permeability of the hematoencephalic barrier.

On subcutaneous injection of atropine in a dose of 1.5-2 ml of a 0.1% solution, in 12 out of the 15 patients under observation a fall in the permeability of the hematoencephalic barrier was found. The percentage content of P^{32} in the cerebrospinal fluid fell from 10-11 to 4-5%, remaining at this level for almost the whole of the rest of the investigation.

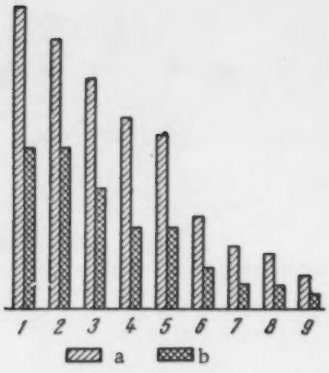

Fig. 3. Distribution of P^{32} in various areas of the brain. a) In cerebrospinal trauma; b) in cerebrospinal trauma after treatment with pentamine. 1) Hypophysis; 2) the ventricular plexi of the brain; 3) contused areas of the cerebral cortex; 4) areas of the cerebral cortex in which there were small hemorrhages with no damage to the vessel walls; 5) diencephalic region; 6) cerebral cortex (intact); 7) subcortical ganglia; 8) brain stem; 9) white matter.

When the patients were injected with phenylephrine (metasympatol) in a dose of 1.5-2 ml of a 1% solution, 9 of the 12 patients under observation showed a fall in the permeability to P^{32}, but it was shorter in duration and less pronounced, while in 2 patients the permeability was unaffected and in one it was increased to 5-7%. The action of atropine and phenylephrine also lowered the acetylcholine content of the cerebrospinal fluid, which evidently led to a fall in the permeability of the hematoencephalic barrier on account of the reduced activity of the tissue hyaluronidases.

In patients with closed head injuries a series of investigations was performed in which 1.5-2 ml of a 0.05% solution of prostigmin was injected. In moderate and severe trauma to the skull, prostigmin lowered the permeability and reduced the acetylcholine content of the cerebrospinal fluid; in slight trauma to the skull, when a low acetylcholine content was observed in the cerebrospinal fluid, prostigmin had no effect, or in some cases even increased the permeability of the hematoencephalic barrier.

Considering that in cerebrospinal trauma there is damage to brain tissue, accompanied by liberation of histamine (I. L. Vaisfel'd), we injected benadryl into patients with acute head injuries, but no noticeable effect was found on the permeability of the hematoencephalic barrier.

The most obvious action on the permeability of the hematoencephalic barrier in patients with cerebrospinal trauma was shown by intramuscular injection of the ganglion-blocking drug pentamine in a dose of 50 mg. In 25 of the 30 patients under observation a fall in the P^{32} content of the cerebrospinal fluid was observed, from 10-20 to 2-5%. In 3 patients the permeability was unchanged and in 2 patients a small rise in the permeability of the hematoencephalic barrier was detected.

After administration of pentamine there was also a considerable fall in the acetylcholine content and the hyaluronidase activity of the cerebrospinal fluid. We obtained analogous results also in experiments on rats.

A brain examination was made on two groups of patients who died two days after severe skull injury; the first group had received P^{32} and repeated injections of pentamine, the second had not. The P^{32} content in various areas of the brain was 2-3 times smaller in the group that had not received pentamine.

We considered it possible to compare these findings, since the results obtained after the injection of pentamine considerably exceeded the limits of variation in patients not treated with pentamine (Fig. 3).

Systematic treatment of patients with cerebrospinal trauma with pentamine gave good clinical results and the electroencephalogram was more rapidly restored to normal than with other forms of therapy.

SUMMARY

The permeability of hematoencephalic barrier was investigated in 100 patients with acute craniocerebral trauma by comparison of the content of P^{32} in the blood and in the cerebrospinal fluid. The content of acetylcholine and the activity of hyaluronidase in cerebrospinal fluid was also studied. These examinations were carried out prior to and after the action of anticholinergic (atropine), adrenergic (phenylephrine), anticholinesterasic (prostigmin), ganglion-blocking (pentamine) and antihistaminic (benadryl) substances. All the substances except benadryl decreased the permeability of the hematoencephalic barrier and the content of acetylcholine in the cerebrospinal fluid. Pentamine produced the most pronounced effect.

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THE EFFECT OF PARTIAL AND TOTAL EXTIRPATION OF THE CEREBRAL CORTEX ON THE "MECHANICAL SECRETION" OF GASTRIC JUICE

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As can be seen from the correspondence of I. P. Pavlov with S. I. Chechulin, I. P. Pavlov not only agreed with the stimulatory role of a mechanical factor (which he had earlier denied) [6] in gastric secretion, but also indicated ways of future investigation of this problem: "Because your fact was hidden from me so well and for so long, more and more tests of all kinds now need to be performed, for example, is not standing in the apparatus a conditioned stimulus that slowly develops its action; is the reflex not concerned with time, and so on . . . ."***

Thus in I. P. Pavlov's view, in order to accept finally the intrinsic importance of the mechanical factor as a stimulus of gastric secretion, the most important consideration was to exclude all the purely conditioned reflex factors which could in some way hide the other mechanisms lying at the basis of this phenomenon.

Pavlov's view was particularly clearly and succintly expressed in the following letter to S. I. Chechulin. "Well," asked Pavlov, "haven't you received your dogs without cerebral hemispheres yet to solve your elusive problem of the mechanical stimulation of gastric juice? Best of luck! Yours, I. Pavlov."****

However, the experiments were not carried out, for Prof. S. I. Chechulin died.

Our aim was to continue these investigations along the lines suggested by I. P. Pavlov [7, 8, 9, 10].

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* In Russian.

** Original Russian pagination. See C. B. translation.

*** Letter of September 7, 1933.

**** Letter dated January 21, 1934. Published in Fiziol. Zhur. SSSR No. 5, 626-627 (1954).

EXPERIMENTAL METHOD

In experiments of long duration on 19 cats and 5 dogs with gastric fistulae the mechanical secretion of gastric juice was investigated in normal conditions and also with unilateral (7 cats, 1 dog) or total (12 cats, 4 dogs) extirpation of the cerebral cortex.

As a control, animals in which the cerebral cortex had not been removed were used, but only the first stage of the procedure was carried out: incision with extensive trauma to the skull.

As a mechanical stimulus to the stomach, we used (with the cats) a rubber balloon: it was introduced through the fistula into the stomach and inflated to a volume of 25 ml; in the dogs we used a rubber tube 75 cm long and with an external diameter of 1.5 cm.

"Mechanical secretion" was studied on an empty stomach (after starvation for 20-21 hrs), with an alkaline reaction of the gastric juice. The volume of gastric juice was estimated at intervals of 15 min and 1 hr; its acidity was determined by titration with decinormal alkaline solution, and its digestive power by means of Mette's albumin tubes (digestion in an incubator for 20 hrs at 37-38°C).

In solution of the problem posed by I. P. Pavlov, the results obtained in the first few days after decortication are of particular interest, i. e., in the period when all the workers in this field (I. P. Pavlov, E. A. Asratian, D. S. Fursikov, B. I. Baiandurov, N. T. Shutova, N. F. Popov, and others) observed not only total loss of the old conditioned connections but also the impossibility of formation of new ones.

In the first 5-10 days after decortication and also after trauma (control experiments) the cats and dogs showed spontaneous secretion of gastric juice, and in the decorticated animals it reappeared during the last 3-4 days before death.

EXPERIMENTAL RESULTS

Experiments performed against a background of spontaneous secretion on the 1st, 2nd and 3rd day after operation beyond doubt, even in animals in a state of severe postoperative shock, that after bilateral decortication the mechanical factor retains its physiological importance. This was shown by the total increase in the volume of gastric juice and by the rise in its acidity and digestive power during mechanical stimulation of the stomach in the decorticated animals, and by the return of the secretion to its initial level when the stimulus was removed from the stomach.

On the cessation of spontaneous secretion and the establishment of an alkaline reaction of the juice of the empty stomach, a further most important index of gastric secretion could be considered – the latent period of stimulation.

Experiments set up on subsequent days (period of observation up to 6 months) with mechanical stimulation of the stomach showed an ever-increasing secretion of gastric juice which, in the majority of cases observed, became transformed into a well-marked hypersecretion. The volume of gastric juice secreted during a 3-hr period of mechanical stimulation in some of the decorticated animals was more than 2-3 times as great as in control experiments (Figs. 1 and 2).

The length of the latent period, normally very labile, was noticeably shortened, and in some experiments was not longer than 5 or even 3 min. The acidity and digestive power of the gastric juice, expressed as average values, either varied within the limits of the original basic level, or in some cases exceeded it, as was shown particularly clearly in the days preceding the death of the animal.

We succeeded in discovering one further relationship demonstrating the extremely increased excitability of the neuro-glandular apparatus of the stomach in the decorticated animals: the process of extinction of gastric secretion was very greatly prolonged – to 2, and sometimes 3 hrs (Fig. 3), while in the normal animal cessation of mechanical stimulation of the stomach leads even after 15-30 min to a sharp fall in gastric secretion and to the establishment of an alkaline character of the gastric juice.

The increased secretory activity of the glandular cells of the stomach in the decorticated animals is also confirmed by histological examination of the gastric mucosa, especially of the lesser curvature of the stomach. This showed that the glands of the fundus of the stomach are characterized by signs of hypersecretion. A consider-

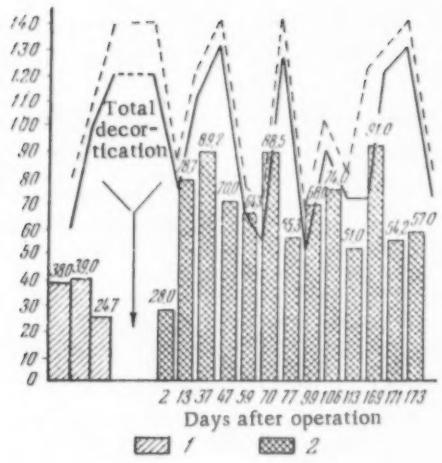

Fig. 1. Changes in the "mechanical secretion" of cat No. 14 after total decortication. 1) Control experiments; 2) main experiments. Each column – total secretion (in ml) during 3 hrs of stimulation. Curve of acidity during the last hour of stimulation. ---) Total acidity; ——) free hydrochloric acid. The mechanical stimulus – a rubber balloon introduced into the stomach.

able number of parietal cells with acidophilic protoplasm, filled with secretory granules are found in them. They are easily distinguishable from the chief cells.

As a rule the secretory activity of the stomach was disturbed only in the decorticated animals and not in the controls.

The brain of the decerebrate animals was carefully examined macroscopically and microscopically after death (stained by Nissl's, van Gieson's or Foot's method or with hematoxylin-eosin).

We carried out the morphological examination of the brain of the decorticated animals in collaboration with the pathologists A. S. Iontov and V. K. Skobnikova.

Examination of the residual areas of the brain of the decorticated cats and dogs showed that only the mediobasal areas of the cerebral hemispheres remained, and in the deeper portion of the brain the boundary of the corpus callosum was preserved. The nerve cells of the cortex lying close to scar formation were in a markedly abnormal state. In the cortex at a distance from the scar the nerve cells appeared swollen. The majority of them showed the picture of vacuolization, tigrolysis and edematous dystrophy. Considerable changes in the giant pyramidal Betz cells led to disturbance of the architec-

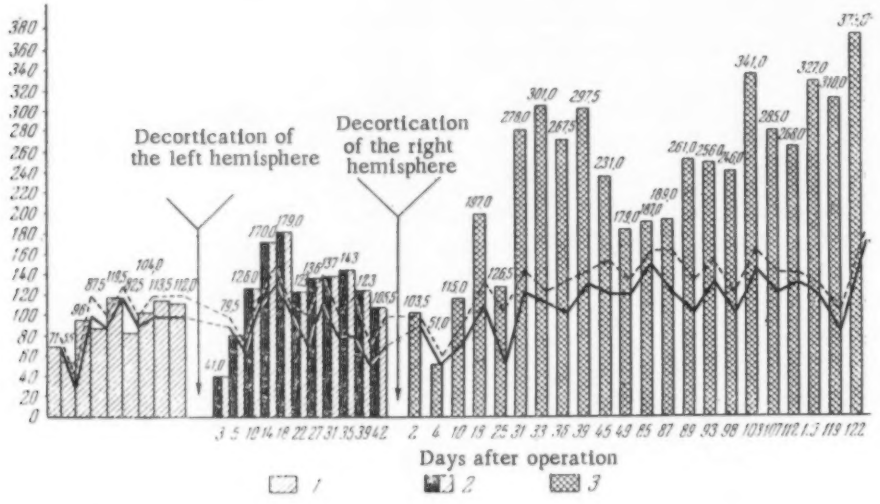

Fig. 2. Changes in the "mechanical secretion" after partial and total decortication of the dog Ryzhia in an experiment in which the mechanical stimulus was a rubber tube 75 cm long and 1.5 cm in diameter, introduced into the stomach. 1) Control experiments; 2) unilateral decortication; 3) bilateral decortication. Each column – volume of secretion (in ml) during 3 hrs of stimulation. ---) Total acidity; ——) free hydrochloric acid.

tonic structure of Area 5. These pathological changes in the nerve cells develop on a basis of severe congestion and edema of the brain not only near the scar but also in the more remote areas (thalamus, caudate nucleus, etc.).

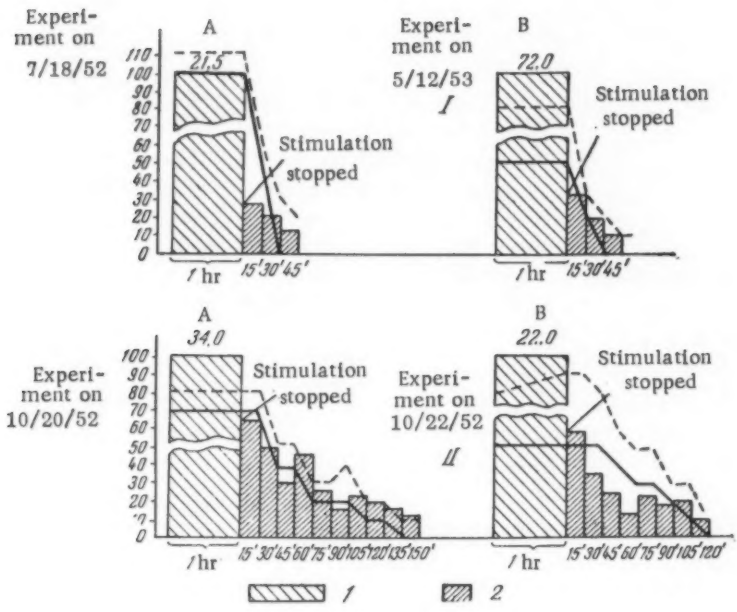

Fig. 3. Rate of cessation of gastric secretion after removal of the mechanical stimulus from the stomach. I) Inthe intact animal; II) after extirpation of the cerebral cortex. A) In cat No. 14; B) in the dog Mika. 1) Volume of secretion (in ml) during one hour of mechanical stimulation; 2) volume of secretion (in ml) after removal of the mechanical stimulus. ---) Total acidity; ——) free hydrochloric acid.

Thus we see that in consequence of operative interference persistent disturbances of the circulation of the blood and cerebrospinal fluid have developed, on which is superimposed the process of scar formation.

As shown by examination of the surviving areas of the cortex, the pathological changes in the neurons were the most pronounced, shown by various forms of dystrophy, leading in some cases to death of the neurons.

The whole of the pathohistological picture of the residual areas of the brain as thus described proves the considerable functional depression of cortical activity, and sometimes its complete suppression. This presumably accounts for the decline in the digestive conditioned reflex reactions to the sight and smell of food which we observed in our decorticated dogs.

Analysis of the experimental data leads us to conclude that the mechanical factor in gastric secretion retains its physiological importance even after the total anatomical and functional exclusion of the cerebral cortex. Since according to the generally accepted view the power of conditioned reflex activity in decorticated animals is irreversibly lost (which our experiments confirm), it may be suggested that the stimulation and increase of secretion of juice following mechanical stimulation of the stomach in decorticated animals takes place by an unconditioned reflex mechanism, as a result of an increase in the tonus of the subcortical vegetative centers, or in other words by subcortical disinhibition.

The phenomenon of "mechanical secretion," first established by us in decerebrate animals, suggests its unconditioned reflex origin. This in itself provides a positive answer to the problem of the physiological importance of the mechanical factor in gastric secretion.

Thus in seeking to solve the difficult problem posed by I. P. Pavlov on the mechanisms responsible for "mechanical secretion" in the normal animal, we came to the conclusion that the main part in its production is played by an unconditioned reflex, activated from the mechanoreceptors of the stomach through a reflex arc

in the vagus nerve center in the medulla oblongata. These findings confirm once more the correctness of the views of S. I. Chechulin [11, 12] that "mechanical secretion" has an intrinsic physiological importance, appearing and becoming consolidated in the animal in the process of phylogenesis.

However, it would be wrong to think that in normal physiological conditions the cerebral cortex has no regulating influence on "mechanical secretion." Experiments in which mechanical stimulation of the stomach was carried out after unilateral extirpation of the cerebral cortex showed that normalization of the "mechanical secretion" in the majority of cases takes place through a stage of hypersecretion (see Fig. 2). In animals with bilateral extirpation of the cerebral cortex it is not possible to observe normalization of the "mechanical secretion," and they die with a well-marked hypersecretory reaction (see Figs. 1 and 2). Evidently the disturbance of the secretory activity of the stomach may be regarded as the result of depression of the regulatory function of the cortex in relation to the activity of the lower centers of the nervous system. This hypothesis is also confirmed, in our opinion, by the rapid normalization of gastric secretion in partial extirpation of the cerebral cortex.

SUMMARY

The author studied the secretion of the gastric juice in response to mechanical stimulation of the stomach both in normal conditions and in partial and complete extirpation of the cortex of the large cerebral hemispheres. Complete extirpation of the cortex of the large cerebral hemispheres did not arrest the "mechanical secretion."

On the contrary, in a majority of cases it caused a pronounced hypersecretory reaction. The latter is manifested in a sharp decrease of the latent period, rise in the amount of gastric juice, as well as by a certain increase of acidity and the digestive power of the mechanically-induced gastric secretion. This hypersecretion of the gastric juice may be considered to be the result of loss of the controlling cortical function on the activity of the subcortical autonomic centers.

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THE STUDY BY THE METHOD OF VITAL STAINING OF THE SORPTIVE PROPERTIES OF CERTAIN ORGANS IN EXPERIMENTAL TUBERCULOSIS

COMMUNICATION II. INVESTIGATION OF THE SORPTIVE PROPERTIES OF SKELETAL MUSCLE

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(Received June 27, 1957. Presented by Active Member Acad. Med. Sci. USSR, D. N. Nasonov*)

Analysis of the physiological condition of muscle tissue cells in tuberculosis is of definite interest on account of the fact that a number of workers believe that muscle possesses particular resistance to tuberculous infection. In a small number of papers devoted to the study of the state of the striated musculature in tuberculosis there are indications of gross pathohistological changes discovered at necropsy in advanced cases of tuberculosis with extensive spread in the internal organs [1, 5, 6, 8, 14]. Only a few isolated papers [10, 12] have been devoted to the experimental study of the early changes in muscle tissue. This is presumably due to the fact that the pathohistological method alone is inadequate for the study of this problem. The purpose of the present investigation was to study the functional state of the muscle tissue in experimental tuberculosis in guinea pigs. In this work we used the method of vital staining devised by D. N. Nasonov.

This method was used by several workers to study the functional state of the skeletal muscles after various forms of trauma [2, 3, 4, 7, 9, 11, 13, 15].

EXPERIMENTAL METHOD

The study of the sorptive properties of the skeletal muscle cells of guinea pigs was made in three series of experiments: after infection of the animals with a virulent culture of bovine tuberculosis, after infection of the animals with an attenuated culture of tuberculosis – BCG, and after infection of the animals with a virulent culture of bovine tuberculosis following preliminary two-stage immunization. In this way three gradations of tuberculosis were produced in the experimental animals, which facilitated the solution of the problem.

The experimental conditions, the method of investigation and also the principle used in drawing up the curves of sorption were described in the first report of an investigation of the sorptive power of brain tissue in infection with tuberculosis.

The experiments were performed on the soleus muscles of both hind limbs. After careful preparation of the muscles the tendons were divided on both sides and the muscles transferred in a resting state to Ringer solution for 10-15 min. Before staining the muscles from both sides were attached by the ends of their tendons to specially curved glass rods, separately, and suspended in a solution of neutral red (0.01%) at a temperature of 18-20°C. After 30 min the muscles were taken out and rinsed in Ringer solution, and the tendons, remains of fatty tissue and fascia removed. After this, the muscles were placed for 24 hrs in a tube with a known volume of 70% alcohol, acidified with a 2% solution of sulfuric acid in order to extract the dye.

A study of the special features of vital staining of muscle tissue was carried out at the same intervals of time after infection as in the investigation of the tissue of the cerebral cortex. In the first series of experiments the animals were killed after 1, 2, 4, 7, 10, 14, 21, 30, 50 days, and in the second and third groups of experiments after 1, 2, 4, 10, 30, 50 days.

* Deceased.

Combination of the Dye with the Soleus Muscles of Healthy and Tuberculous Guinea Pigs

Conditions of infection	No. of expts.	Period of infection							
		1 day	2 days	4 days	7 days	10 days	14 days	21 days	30 days	50 days
TB	10—30	—23.8±4.6	—16.2±2.8	—15.3±2.5	—20±4	+3.4±1.4	+27±1.8	+33.2±4.7	+24.2±5.5	+14.7±3.1
BCG	3	—16±0.58	—13.3±3.7	—13±1	—	+0.7±0.33	—	—	+12±2.67	+8.7±3.35
BCG + TB	3	—22.3±1.85	—16±2.51	+1.3±4.05	—	+5.3±1.2	—	—	+14.7±5.04	+10±4.51

Note. The signs (+) and (−) indicate an increase and decrease in the quantity of dye combined with the soleus muscles of the experimental animals in percentages of the quantity of dye combined with the soleus muscles of control animals. The results are shown in the form of the arithmetic mean of the total number of experiments.

Curves showing the changes in the sorptive power of the soleus muscles of a guinea pig in experimental tuberculosis. Along the axis of the abscissas — time after infection (in days); along the ordinate axis — amount of combined neutral red, expressed as a percentage of the amount of neutral red combined with the muscles of the control animals: A) in animals infected with a virulent culture of bovine tuberculosis; B) in animals infected with an attenuated culture of tuberculosis, BCG; C) in doubly immunized animals infected with a virulent culture of bovine tuberculosis.

Altogether 400 experiments were made in the first series, and 40 each in the other two series.

Meanwhile at the same times histological examinations were made of the internal organs and skeletal muscles (2 animals for each period of time). For this purpose the hematoxylin-eosin and picrofuchsin methods were used to stain the organs.

In order to estimate correctly the changes in the sorptive properties of the muscles in the experimental animals it was essential first of all to find out how the dry weight of the muscles altered during the development of the disease. Investigations showed that in all 3 series of experiments the dry weight of the muscles varied only insignificantly, particularly with infection by BCG and by a virulent culture when the animals were immunized, which leads us to consider that our results obtained from the study of the changes in the sorptive properties are reliable in all stages of the infection.

EXPERIMENTAL RESULTS

On histological examination we were unable to find any specific morphological changes in the muscle tissue of the animals in all three series of experiments at all stages of development of tuberculosis.

In studying the sorptive properties of the muscles in the three series of experiments we observed a change in the degree of combination of dye, which was biphasic in character. The first phase was characterized by a fall and the second by a rise in the sorptive power. We observed an analogous phenomenon when studying the changes in the sorptive power of the cerebral cortex.

The duration of the first phase in the three series of experiments was not the same. The longest duration of this phase was observed in the first series of experiments with infection of the animals by a virulent culture of Koch's bacilli (see figure and table).

The degree of lowering of the sorptive power in the first phase after infection with a virulent culture of unimmunized animals and also of those previously immunized was the same, while after infection with a culture of BCG there was a less pronounced fall in the sorptive power.

Variations in the sorptive level were most clearly expressed after infection of the animals with a virulent culture of Koch's bacilli.

The second phase of the reaction – a rise in the sorptive power of the muscles – was also unequal in all three series of experiments. The quantity of combined neutral red in all the groups of experimental animals in this phase was from 12-33% greater than that found in the control group, and moreover the greatest degree of change in the functional state of the muscle tissue was observed after infection of guinea pigs with a virulent culture of Koch's bacilli. In this case these changes reached their maximum much sooner (on the 21st day) than in the other two series of experiments (on the 30th day after infection).

In all the series of experiments, after the second phase of the reaction a fall in the sorptive power of the muscles was observed, although it was still higher than in the control animals and remained so until the terminal period.

In this period, when the second phase of change in the sorptive power of the muscles was observed, the tuberculous process was progressing unrestrainedly in the internal organs.

The experiments which we performed showed that even within a few hours of infection with tuberculosis, the guinea pigs showed changes in the functional state of the muscle tissue.

As the disease progresses, with development of severe specific morphological changes in the internal organs, the sorptive power of the muscle cells rises intensively. This indicates the intensification of paranecrosis and alterative processes in the sarcoplasm of the cells. The muscle tissue at this period undergoes profound changes, as shown by the alteration in its sorptive properties. With the development of the disease irreversible degenerative changes arise in the cell structures of all the organs, including the muscles. This is reflected in the character of the sorptive power of the cells.

Thus our investigation of the functional state of the muscle cells by the method of vital staining showed that these cells in experimental tuberculosis are actively involved in the reaction of the body within a few hours of infection. Analogous findings were observed after vaccination with a BCG culture.

SUMMARY

The method of vital staining was used in experimentally-induced tuberculosis for the study of the physiological condition of the cells of the cross-striated muscle.

It was established that at the early stages of this infection when no morphological changes are, as yet, present in internal organs, substantial changes take place in the cells of the skeletal muscles. These changes are reflected in the change of the sorptive properties of the cellular sarcoplasm.

The dynamics of the sorptive power is not the same in all cases but has a regular wave-like biphasic character. The sorptive power of the cells is intensively increased with increase of the morphological changes in internal organs.

The method of vital staining demonstrated that the cells of the skeletal muscle do not remain intact and are actively involved in the reaction of the organism to tuberculous infection.

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THE SELECTIVE EFFECT OF SOME CHEMICAL STIMULANTS ON THE REFLEXES FROM CHEMORECEPTORS

COMMUNICATION VIII. THE EFFECT OF STREPTOMYCIN ON REFLEXES FROM THE CHEMORECEPTORS AND MECHANORECEPTORS OF THE PERICARDIUM

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In previous reports [1, 3, 4, 5] it has been shown that the parenteral injection of streptomycin depresses reflexes from the chemoreceptors of the small intestine, spleen, kidneys and the tissues of the hind limb.

According to reports in the literature, the conducting pathways from the chemoreceptors of the zones mentioned above pass through the spinal cord [2, 8, 9, 11]. In this connection it is important to ascertain the character of the effect of streptomycin on the reflexes from those reflexogenic zones from which the conducing path-

* In Russian.

ways lead mainly direct to the medulla oblongata, missing the spinal cord. A zone of this sort, in particular, is the interoceptive zone of the pericardium. As shown by a number of workers [2, 6, 7, 8], the main bulk of afferent impulses from the receptors of the pericardium reach the central nervous system by the aortic and vagus nerves, missing the spinal cord.

EXPERIMENTAL METHOD

Experiments were performed on cats under urethan anesthesia. The level of the arterial pressure and the respiratory movements were recorded by the usual methods.

In order to study the reflexes from the receptors in the pericardium, Drinker's method was used [10].

As a stimulant of the pericardial chemoreceptors we used nicotine in a dilution of 10^{-9} to 10^{-3}, which was injected in a volume of 0.3-0.5 ml into the pericardial cavity, and then washed out with warm physiological saline. In each experiment several dilutions of nicotine were used. The mechanoreceptors of the pericardium were stimulated by a stream of warm physiological saline directed at the pericardial membranes always in the same force and volume. Reflexes from the chemo- and mechanoreceptors of the pericardium were in most cases shown in the form of depression of the arterial pressure, increase in the rate of respiration and in its amplitude. Less commonly there was an increase in the level of the blood pressure.

In some of the experiments the threshold of excitability of the tibial nerve to stimulation with an electric current was determined in addition. Streptomycin (sulfate or calcium chloride complex) was in most cases injected intravenously in a dose of 70,000-127,000 units, but in some cases was injected intramuscularly in a dose of 250,000-400,000 units. After 5-20 min, and then after each 10-20 min repeated examinations were made of the excitability of the receptors of the pericardium to chemical and mechanical stimuli under the influence of the antibiotic.

EXPERIMENTAL RESULTS

The effect of streptomycin on the reflexes from the receptors of the pericardium was studied in 12 experiments.

In 2 experiments the effect of the antibiotic (400,000 units intramuscularly) was studied on the reflexes from the chemoreceptors of the pericardium to nicotine in a dilution of $1:10^{-9}$. In both cases the magnitude of the original reflex (depressor) gradually diminished, and one-and-a-half hours after the injection of streptomycin the reflex to nicotine was found to be completely suppressed (Fig. 1).

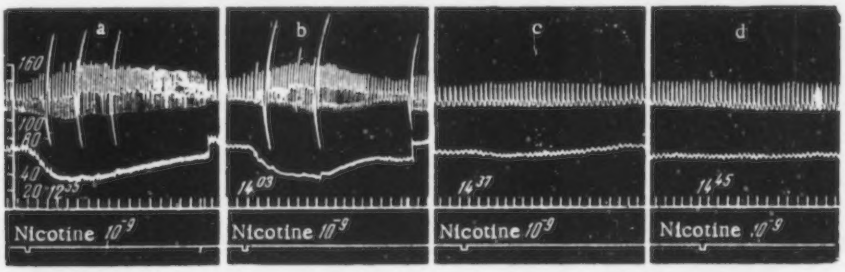

Fig. 1. The effect of streptomycin on reflexes from the chemoreceptors of the pericardium caused by nicotine. a) Original reflex; b, c, d) gradual development of complete suppression of the reflex as a result of streptomycin injected intramuscularly in 13 hrs in a dose of 400,000 units. Interpretation of the curves (from above downwards): respiration, arterial pressure, time marker (5 sec), stimulation marker.

In 3 experiments the effect of streptomycin was studied on the reflexes from the chemoreceptors of the pericardium caused by nicotine in a dilution of $1:10^{-8}$. One hour after the intravenous injection of streptomycin the reflex was distorted. In 2 experiments after injection of the preparation intramuscularly the reaction to nicotine diminished to 60 and 63% (after one-and-a-half hours).

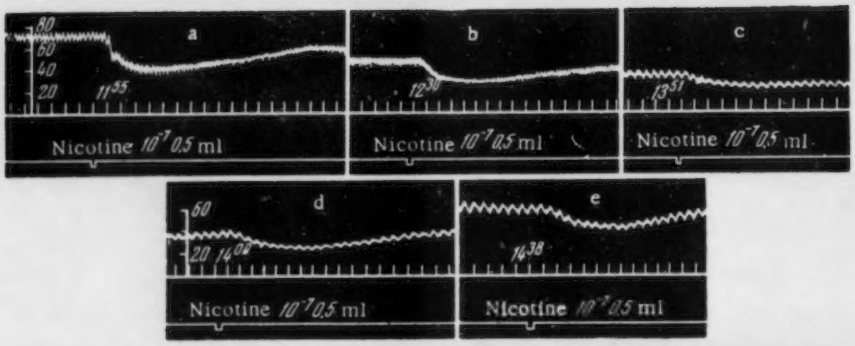

Fig. 2. The effect of streptomycin on reflexes from the chemoreceptors of the pericardium caused by injection of nicotine in a dilution of $1:10^{-7}$ (0.5 ml). a) Original reflex; b, c, d, e) suppression and subsequent partial restoration of the reflex after administration of streptomycin (injected intravenously in 12 hrs 14 min in a dose of 100,000 units). Interpretation of the curves (from above downwards): blood pressure, time marker (5 sec), stimulation marker.

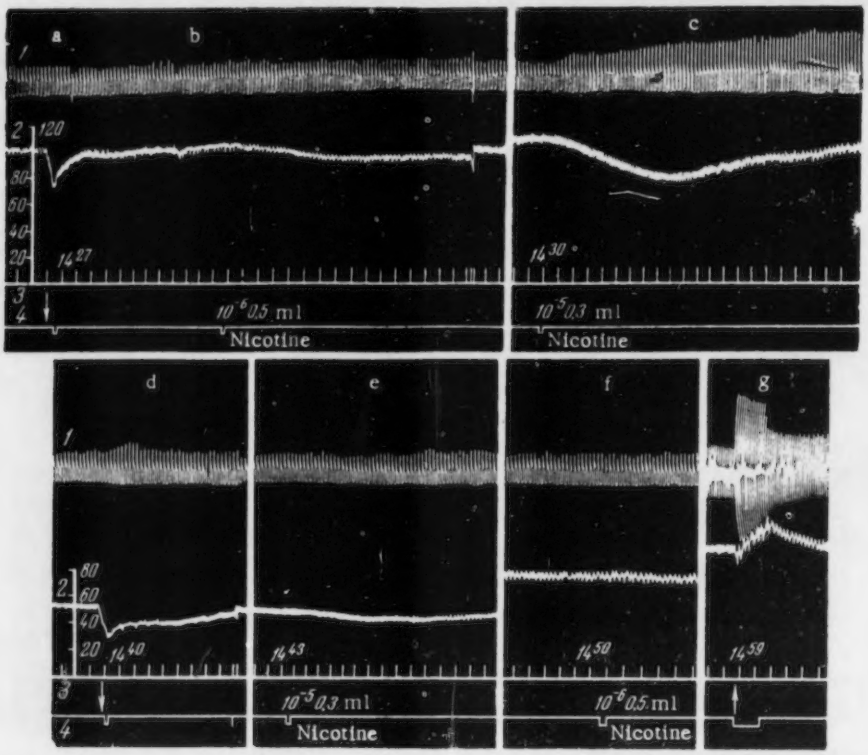

Fig. 3. The effect of streptomycin on reflexes caused by stimulation of the mechano- and chemoreceptors of the pericardium. a) Reflex caused by stimulation of the mechanoreceptors; b) weak reflex caused by stimulation of the chemoreceptors (nicotine $1:10^{-6}$, 0.5 ml); c) clearly marked reflex caused by stimulation of the chemoreceptors (nicotine, $1:10^{-5}$, 0.3 ml). Tracings a, b and c were made before the injection of streptomycin. The next tracings d, e and f were made after the injection of streptomycin. The reflex caused by stimulation of the mechanoreceptors (d) was preserved. The reflexes caused by nicotine are absent; g) reflex caused by stimulation (↑) of the peroneal cerve by an electric current. Interpretation of the curves as in Fig. 1.

In 7 experiments the effect of injection of streptomycin was studied on the reflex caused by nicotine in a dilution of $1:10^{-7}$. After the administration of the antibiotic the reflex to nicotine was completely suppressed in 2 experiments, and in 4 cases the reflex was considerably weakened (Fig. 2).

In 7 experiments the effect of streptomycin was studied on the reflex caused by nicotine in a dilution of $1:10^{-6}$. Under the influence of streptomycin the original reflex completely disappeared or was distorted (became pressor instead of depressor) in 4 cases, and in the remaining experiments it was considerably reduced. The maximum suppression of the reaction to nicotine was observed in 4 experiments at the beginning of the test – from 10-20 min after injection of the antibiotic. In the remaining experiments this effect began somewhat later – after 40-60 min.

In 4 experiments the effect of streptomycin was studied on the depressor reflex caused by nicotine in a dilution of $1:10^{-5}$. After intravenous injection of the antibiotic the original reaction was reduced in every case, although to a lesser degree than after administration of nicotine in higher dilutions.

In 9 experiments the effect of streptomycin was studied at the same time on the reflexes from the chemoreceptors (the results of these observations have been given above) and from the mechanoreceptors of the pericardium.

The original reflexes from the mechanoreceptors of the pericardium were depressor in every case. Under the influence of streptomycin in 6 experiments the reflexes from the mechanoreceptors of the pericardium diminished slightly, while in 3 experiments they increased. Thus in the majority of cases streptomycin has no obvious suppressive effect on the reflexes from the mechanoreceptors of the pericardium. At the same time the reflexes from the chemoreceptors were severely suppressed (Fig. 3). The different resistance to streptomycin of the reflexes from the chemo- and mechanoreceptors will be even more obvious if we follow the progress of the changes in the reflexes from the mechanoreceptors of the pericardium during the period of action of the antibiotic. In the majority of cases suppression of the reflexes from the mechanoreceptors of the pericardium was transient, and the reflex quickly regained its original magnitude or even exceeded it.

The different effects shown by streptomycin on the reflexes from the chemoreceptors and mechanoreceptors of the pericardium did not depend on differences in the magnitude of the original reflexes. Thus in the experiments in which the reflex fall in pressure on stimulation of the mechanoreceptors of the pericardium was from 6-9 mm Hg, under the influence of the antibiotic it either increased or briefly and insignificantly diminished. In contrast to this, the reflexes from the chemoreceptors to nicotine, which were far more pronounced, were suppressed completely or reduced by streptomycin.

In order to discover the nature of the suppressive influence of streptomycin on the reflexes from the chemorectpors of the pericardium, in some of the experiments at the moment of greatest suppression of these reactions the threshold of excitability of a peripheral somatic sensory nerve (the tibial) to stimulation with an electric current was determined.

It was established that at the period when the excitability of the vasomotor center to impulses from the chemoreceptors of the pericardium was shown to be considerably suppressed by the effect of the antibiotic, its excitability to impulses from the tibial nerve still remained sufficiently high (Fig. 3,g).

In experiments in which the chemo- and mechanoreceptors of the pericardium were stimulated, not only vasomotor reflexes but respiratory reactions were also observed,and in most cases these took the form of increased amplitude and rate of the respiratory movements. Under the influence of streptomycin the reflexes acting on respiration were changed most commonly parallel with the reflexes acting on the blood pressure. In some cases it could be seen that the reflexes from the chemoreceptors on respiration were suppressed more quickly and intensively than those acting on the blood pressure.

The results of the observations carried out prove that streptomycin injected parenterally exhibits a marked suppressive action on the reflexes from the chemoreceptors and a much less pronounced action on the mechanoreceptors of the pericardium. From the results obtained it follows that streptomycin suppresses the reflexes from the chemoreceptors also in the case where the afferent pathways pass mainly direct to the medulla oblongata, missing the spinal cord.

The observations quoted in the present paper confirm yet again the hypothesis which we put forward previously that streptomycin suppresses reflexes from the chemoreceptors as a result of the effect which it exerts on the afferent part of the reflex arc in the central nervous system, commencing with the chemoreceptors.

SUMMARY

Short-term experiments were performed on cats under urethan anesthesia. The effect of streptomycin administered parenterally on the reflexes from the pericardial chemoreceptors and mechanoreceptors was studied. Intravenous injection of streptomycin in the dose of 250,000-400,000 units and in intramuscular injection of 70,000-127,000 units completely depresses or considerably inhibits the reflexes from the pericardial chemoreceptors caused by nicotine. However, in the majority of experiments no depression of reflexes appearing in stimulation of pericardial mechanoreceptors was noted in similar experimental conditions. At times only an insignificant decrease of these reflexes was revealed.

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COMPARISON OF THE ACTIVE CONCENTRATIONS OF SOME ANTIBIOTICS IN THE LYMPH OF THE PERIPHERAL VESSELS AND THE THORACIC DUCT

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The concentration of antibacterial preparations in the body is usually judged by their concentration in the blood. However, the active concentration should be considered to be that which is attained by the preparation in the tissue fluids.

Certain workers judge the concentration of chemotherapeutic drugs in the tissue fluids by their content in the cerebrospinal fluid [3, 4], but the results so obtained do not reflect sufficiently accurately the changes in their active concentrations in the tissue fluid over a period of time. This is explained by the fact that diffusion and the dynamics of the circulation of the tissue fluid and cerebrospinal fluid are brought about by different factors.

Work published in recent years on the study of the diffusion of antibacterial drugs from the blood stream into the tissue fluid was carried out by comparing the active concentrations of these substances in the blood and in the lymph obtained from the thoracic duct [1, 2]. These workers started from the assumption that the concentration of the antibiotics in the lymph of the thoracic duct corresponded to their concentration in the tissue fluids.

However, it is well known that the lymph from the peripheral vessels differs considerably in its composition from the lymph of the thoracic duct: it contains a smaller quantity of proteins, and practically no fibrinogen, therefore it does not clot. The lymph of the thoracic duct is profoundly changed by the influence of many substances entering the lymphatic system from the liver. For this reason many chemotherapeutic drugs, which enter the lymph of the thoracic duct through the liver, must be present here in higher concentrations than those found in the lymph of the peripheral vessels.

Since this question is important in principle, we attempted to study concurrently the active concentrations of certain antibiotics in the lymph of the peripheral vessels (jugular lymphatic trunk) and of the thoracic duct in animals.

EXPERIMENTAL METHOD

Experiments were performed on 15 dogs weighing from 10 to 21 kg. The thoracic duct and jugular lymphatic trunk were exposed under morphine anesthesia. Morphine was injected subcutaneously in a dose of 15 mg per 1 kg body weight of the animal. In the exposed and isolated lymphatic vessels glass cannulas were introduced through which periodically samples of lymph were withdrawn. Blood was taken from the jugular vein.

Investigations were made of the concentration of penicillin, biomycin, tetracycline and terramycin. The preparations were injected intravenously in a single dose: penicillin in a dose of 10,000 units/kg, biomycin, tetracycline and terramycin in a dose of 10 mg/kg. Samples of blood and lymph from the thoracic duct and jugular lymphatic trunk were taken 5, 15, and 30 min after the injection of the drug, and then every 1-2 hrs for 24 hrs. As a control, samples were taken from the blood and lymph of all the experimental animals before the injection of antibiotic.

The active concentrations of penicillin in the lymph and serum were determined by the method of serial dilutions in Hiss' medium with glucose and Andrade's reagent. The test microorganism used was Staphylococcus aureus strain No. 209. The concentration of the antibiotics of the tetracycline group in the serum and lymph was determined by the method of diffusion in agar on Petri dishes with cylinders (test microorganism a spore-bearing culture of L_2).

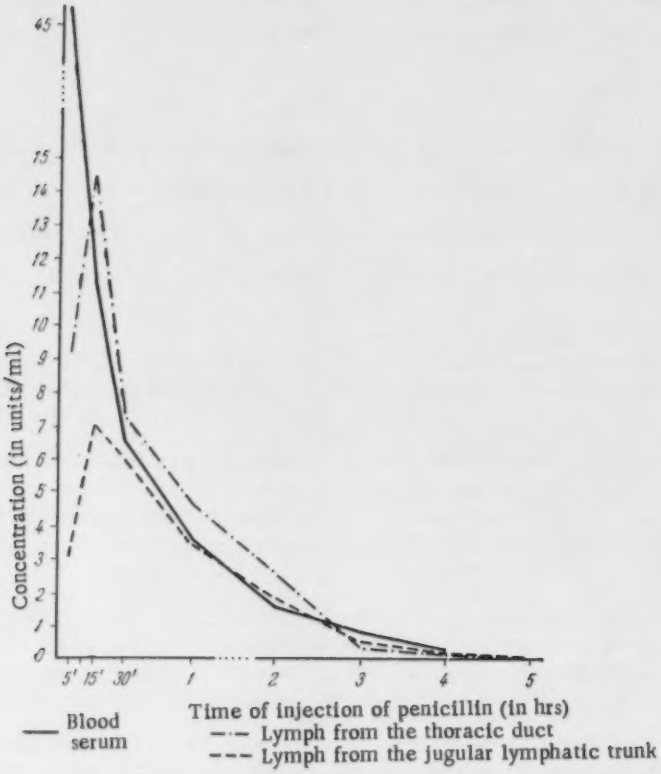

Fig. 1. Concentration of penicillin in the blood serum and lymph from the thoracic duct and jugular lymphatic trunk of dogs after intravenous injection of 10,000 units/kg.

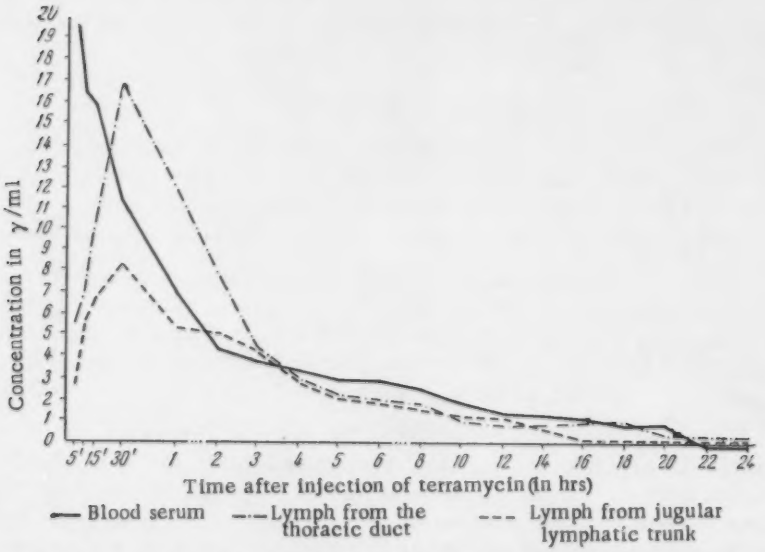

Fig. 2. Concentration of terramycin in the serum and lymph from the thoracic duct and the jugular lymphatic trunk in dogs after intravenous injection of 10 mg/kg.

Summarized Results of a Comparative Study of Active Concentrations of Antibiotics in the Lymph of the Peripheral Vessels and the Thoracic Duct

Time after injection of antibiotic	Penicillin			Biomycin			Tetracycline			Terramycin		
	Concentration, units/ml			Concentration in γ/ml								
	in blood serum	in lymph of thoracic duct	in lymph of jugular lymphatic trunk	in blood serum	in lymph of thoracic duct	in lymph of jugular lymphatic trunk	in blood serum	in lymph of thoracic duct	in lymph of jugular lymphatic trunk	in blood serum	in lymph of thoracic duct	in lymph of lymphatic trunk
5 min	45.4	9.2	3.2	6.2	2.2	0.6	15.0	2.0	1.1	20.0	5.5	2.6
10 »	—	—	—	5.3	3.7	1.3	9.7	6.0	3.4	16.5	7.0	5.5
15 »	11.3	14.4	7.2	3.3	5.5	1.8	9.4	13.3	5.9	15.8	10.0	6.5
30 »	6.6	7.2	6.1	2.6	5.3	1.5	7.5	13.8	4.6	11.3	17.0	8.3
1 hrs	3.6	4.6	3.6	1.8	2.8	1.1	4.7	9.5	4.1	7.0	12.0	5.3
2 hrs	1.6	2.5	1.8	1.3	2.2	0.9	3.2	4.0	2.3	4.5	8.0	5.1
3 »	0.8	0.4	0.5	1.1	1.5	0.6	2.7	3.4	1.6	3.9	4.5	4.3
4 »	0.2	0.1	0.1	1.0	1.3	0.5	2.2	2.8	1.3	3.4	3.0	3.0
5 days	0.05	0.1	0.05	0.8	1.1	0.4	1.8	2.4	1.0	3.0	2.3	2.3
6 »	0	0.01	0.01	0.7	1.0	0.3	1.5	1.7	0.8	2.9	2.1	2.1
8 »	0	0	0	0.6	0.8	0.2	1.2	1.2	0.8	2.5	1.8	1.8
10 »	0	0	0	0.5	0.7	0.1	1.1	1.0	0.7	1.9	1.2	1.3
12 »	0	0	0	0.5	0.6	0.1	0.8	0.8	0.5	1.3	1.0	1.3
14 »	0	0	0	0.3	0.5	0.1	0.6	0.7	0.4	1.3	1.0	0.8
16 »	0	0	0	0.2	0.4	0.05	0.5	0.6	0.2	1.2	1.0	0
18 »	0	0	0	0	0.2	0	0.4	0.4	0.2	0.9	1.0	0
20 »	0	0	0	0	0	0	0.3	0.1	0.1	0.8	0.5	0
22 days	0	0	0	0	0	0	0.05	0.1	0.05	0	0.4	0
24 »	0	0	0	0	0	0	0.05	0.1	0.05	0	0.3	0

Symbols used: 0 — no activity; — no test.

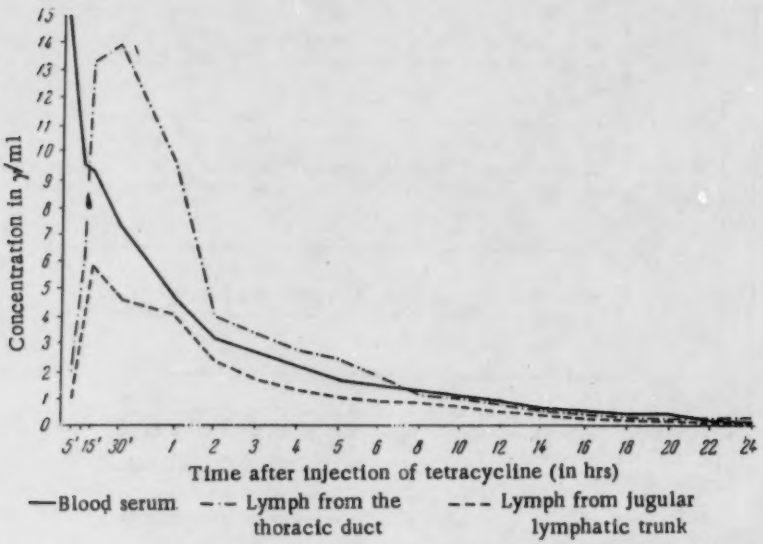
Concentration in γ/ml
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
5' 15' 30' 1 2 3 4 5 6 8 10 12 14 16 18 20 22 24
Time after injection of tetracycline (in hrs)
—Blood serum
– – Lymph from the thoracic duct
– – – Lymph from jugular lymphatic trunk

Fig. 3. Concentration of tetracycline in the serum and lymph from the thoracic duct and the jugular lymphatic trunk in dogs after intravenous injection of 10 mg/kg.

The experimental results are shown in the table.

Changes in the active concentrations of penicillin, biomycin, tetracycline and terramycin in the serum, and lymph from the thoracic duct and the jugular lymphatic trunk are shown in Figs. 1, 2, and 3.

It follows from the table and figures that the greatest concentrations in the lymph from both the thoracic duct and the jugular lymphatic trunk are found from 15-30 min after the intravenous injection of the antibiotics. The active concentrations of all the antibiotics tested were as a rule lower in the lymph from the jugular lymphatic trunk than in the lymph from the thoracic duct. Only in rare cases and in later tests were the concentrations of the antibiotics in the lymph from the jugular trunk and thoracic duct equalized.

It follows from these experiments that the study of the concentration of antibiotics in the tissue fluid of the body must be based on estimations of their concentrations in the lymph of the peripheral vessels and not the lymph of the thoracic duct.

SUMMARY

A comparative study was carried out on the active concentrations of certain antibiotics in the lymph of the peripheral vessels and in the lymph of the thoracic duct. Experiments were performed on dogs. Antibiotics under investigation were introduced intravenously in a single dose (10,000 units/kg penicillin, 10 mg/kg biomycin, tetracycline and terramycin). Active concentrations of all of these antibiotics are as a rule lower in the lymph of the duct of the head than in the lymph of the thoracic duct. Conclusion may be drawn that the study of concentration of antibiotics in tissue fluids of the organism must be based on determinations of their concentration in the lymph of peripheral vessels and not in the lymph of the thoracic duct.

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BIOCHEMISTRY AND BIOPHYSICS

INTERCONVERSION OF LIVER AND SERUM PROTEINS IN HOMOGENATES OF RATS' LIVER

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In a previous paper [4] it was shown that after intravenous injection of rats with isogenous serum proteins labeled with methionine-S^{35}, radioactivity is found in the proteins of the liver, including the insoluble ones, very rapidly. The conversion of serum proteins into liver proteins takes place mainly without the preliminary breakdown of the injected proteins into amino acids. At almost the same time as ourselves, a number of workers [1, 5, 9] from analogous experiments on animals and also with tissue cultures [7, 8, 10, 12], came to the same conclusion.

In order to make a further study of the mechanism of this conversion, it was important to find out how the same process takes place in isolated liver tissue, and in particular in homogenates of the liver.

EXPERIMENTAL METHOD

Experiments were performed on white rats. The proteins were labeled in vivo by injecting the donor rat with 0.5 mC of glycine-1-C^{14}. The labeled serum proteins of the donor were subdivided by the accepted laboratory method of electrophoresis on starch [2] in a veronal buffer (pH = 8.6, μ = 0.18) into 3 fractions: albumins, α-globulins and β + γ-globulins. The soluble liver proteins of the donor were extracted by a method described previously [3], and were also subjected to electrophoresis on starch. As a preparation of "liver globulins" we used proteins whose electrophoretic mobility was close to the electrophoretic mobility of the β- and γ-globulins of the serum. A test of the homogeneity of the serum and liver protein fractions obtained showed, by means of paper electrophoresis, that the serum albumins contained no traces of globulins and that the β- and γ-globulin fractions contained no traces of albumins. The α-globulin fraction of the serum was found to be nonhomogeneous and so was not used for further investigation.

Incubation of the labeled proteins with liver homogenates was carried out in Ringer-bicarbonate buffer (not containing Ca^{++} ions; pH = 7.4) in an atmosphere of 95% O_2 and 5% CO_2 for 2 hrs at 37°C. Before incubation the labeled proteins were dialyzed and the nonprotein label did not exceed 0.6%. The amount of homogenate in each test was equivalent to 1.5 g of liver. The radioactivity of the added proteins varied from 150,000 to 300,000 imp/min per test. To each test, in addition, was added 3 mg of ATP and 10 mg of glucose.

After incubation the proteins were extracted with physiological saline and separated into albumins and globulins as described above. The separated protein fractions were extracted from the starch and precipitated with trichloroacetic acid, and after the usual treatment [4] their radioactivity was determined by means of a gas flow meter.

EXPERIMENTAL RESULTS

As can be seen in Fig. 1, after incubation of the labeled serum albumins with liver homogenates, the radioactivity of globulins extracted from them was markedly higher than before incubation. On adding to the incubation medium a mixture of all the amino acids necessary for protein synthesis, except glycine, the radioactivity in the extracted globulins was considerably increased. The addition of nonlabeled glycine to this mixture (4 mg of the D–L-form per test) reduced the radioactivity, but in this case the radioactivity of the globulins was higher than in the test before incubation. The fall in the radioactivity of the isolated globulins after the addition of unlabeled glycine to the homogenate demonstrates that synthesis of globulins takes place mainly from free amino acids. Nevertheless the fact that in this case the radioactivity remains higher than before incubation gave grounds for considering that part of the labeled serum albumin was converted into liver globulin without undergoing extensive breakdown into amino acids.

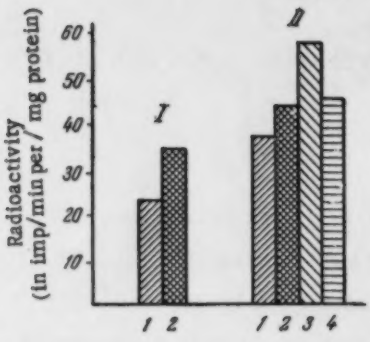

Fig. 1. Radioactivity of the globulins after incubation of liver homogenates from rats with serum albumins labeled with glycine-1-C^{14}. 1) Before incubation; 2) after incubation; 3) after incubation and in the presence of a mixture of the following amino acids: tryptophane, arginine, threonine, lysine, leucine, valine, histidine, isoleucine, phenylalanine, tyrosine, methionine, glutamic acid, aspartic acid, alanine, serine, proline and cystine (at the rate of 2 mg of the L-form of each amino acid per test); 4) after incubation and in the presence of an amino acid mixture including unlabeled glycine (4 mg of the D–L-form). Radioactivity of the added albumins: I) 173,000 imp/min in the test; II) 290,000 imp/min in the test.

Different results were obtained after incubation of labeled serum globulins. As can be seen from Fig. 2, in this case the radioactivity of the albumins isolated after incubation not only was no higher than before incubation, but on the contrary was considerably reduced. This fall may be explained by the fact that the incubated globulins contained a small trace of labeled albumins, undetectable by electrophoresis, which was mixed on incubation with the unlabeled liver albumins. If synthesis of labeled albumins from labeled globulins from the serum did not occur, a mixing of this sort must lead to a fall in the radioactivity of the albumin fraction was A marked increase in the radioactivity of the albumin fraction was observed after incubation of liver homogenates with labeled liver globulins (Fig. 3). Both in this case and in that of incubation of labeled albumins, the addition of unlabeled glycine to the incubation medium slightly reduced the radioactivity, but even so left it higher than that of the albumins before incubation. This shows that the process of formation of serum albumin from liver globulins in small quantities in the homogenates may take place without breakdown to amino acids.

The results obtained, that the serum albumins are far more rapidly converted in liver homogenates than the serum globulins, are in full agreement with the recently published findings of Roberts and Kelley [11], who report that serum albumins added to liver sections are oxidized and utilized for glyconeogenesis much more rapidly than serum globulins.

There are points of disagreement, however, between our findings and those of the authors cited on the mechanism of conversion of serum albumin into globulin. From our findings, as pointed out above, this conversion took place in liver homogenates mainly after breakdown of the albumin to amino acids, with subsequent synthesis of globulins. According to Roberts and Kelley the conversion of albumin which takes place in liver sections does so without breakdown into amino acids. It must also be pointed out that in their latest report, Campbell and Stone [6] came to the conclusion that in vivo conversion of injected serum proteins into liver proteins takes place only after breakdown into amino acids.

Thus in the literature so far there are two opposing points of view on the question of the mechanism of conversion of injected serum proteins into liver proteins. Our own findings persuade us that depending on the conditions present in the body, either the first or the second process may take place. One of the main factors determining the course of this process may be the proteolytic activity of the tissues. If this activity is great, as for example in liver homogenates, synthesis takes place mainly from amino acids liberated as a result of breakdown

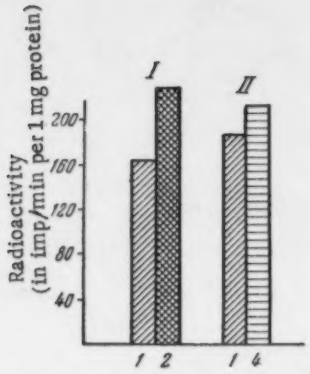

Fig. 2. Radioactivity of albumin after incubation of liver homogenates from rats with serum globulins labeled with glycine-1-C^{14}. Radioactivity of the added globulins in each test 160,000 imp/min. Interpretation as in Fig. 1.

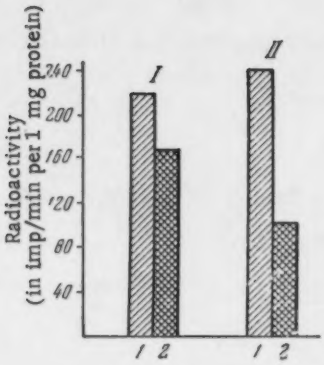

Fig. 3. Radioactivity of albumin after incubation of liver homogenates from rats with liver globulins labeled with glycine-1-C^{14}. Radioactivity of added globulins: I) 163,000 imp/min in the test; II) 230,000 imp/min in the test. Interpretation as in Fig. 1.

of proteins; if however the intensity of proteolysis is not great, then the formation of liver proteins from injected serum proteins takes place mainly by conversion without preliminary breakdown of the injected protein to amino acids.

SUMMARY

It was shown with the aid of proteins labeled by glycine-1-C^{14} in vivo that serum albumin is transformed into globulins in the homogenates of rat's liver. This takes place mostly after disintegration to amino acids of the added albumins. In these conditions hepatic globulins are transformed into albumins. There is no production of albumins from the serum β- and γ- globulins added to the homogenates.

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THE USE OF MITOGENETIC SPECTRAL ANALYSIS FOR DETECTION OF ACETYLCHOLINE IN FROG'S MUSCLE DURING VARIOUS TYPES OF STIMULATION

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The application of mitogenetic spectral analysis to the problem of nerve stimulation has shown that the radiation is a sensitive indicator of the functional condition of the neuromuscular system. This may be judged by the change in its spectral composition attending the most insignificant functional changes [1-4, 6, 7]. These changes in the spectrum, resulting from definite molecular conversions of the substrate of excitation, enable the character of several processes to be elucidated (for example, the degree of dispersion and polymerization of peptides) and their connection with the observed functional changes to be established. For this reason the use of mitogenetic spectral analysis in the detection of acetylcholine in vivo was held to be important, although its part in the stimulation process is the subject of lively discussion in the literature, since it made possible the further study of the link between the metabolism of the mediator and the state of the molecular substrate.

The work, which was performed during the autumn and winter of 1956-1957, consisted of two parts: preliminary experiments to determine the standard spectrum of acetylcholine and experiments to detect acetylcholine in vivo in the muscle of the frog (Rana temporaria, male) by the spectral method. We used a mitogenetic method that has been developed with an enzyme biodetector [4].

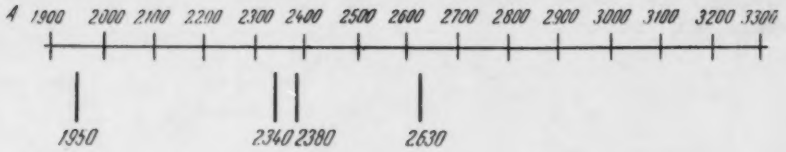
The instability of acetylcholine in solution prompted the suggestion that by analogy with other compounds already studied, it is a source of spontaneous mitogenetic radiation. The preliminary experiments showed that in fact a solution of acetylcholine chloride, prepared freshly, is quite a powerful source of radiation, the maximum effect of which appears in a concentration of $0.5 \cdot 10^{-2}$ with an exposure of 25-30 sec. The radiation from the solution falls off from 5-6 min after preparation.

The spectrum was obtained by means of a spectrograph* A quartz dish containing a freshly prepared solution of acetylcholine was placed in front of the slit of the collimator. The whole field of mitogenetic activity in the ultraviolet (1900-3200 A) was subdivided by means of standard stencils with apertures of 100 A into corresponding areas. The 100 A areas in which the mitogenetic effect was found were subdivided into areas of 20 A by means of monochromatic apertures. Mitogenetically active areas of 20 A were further divided into areas of 10 A and in the same way these were divided into lines 5 A in width. Acetylcholine chloride was used in the experiments, and it was therefore necessary to exclude from the general spectrum the spectral lines of the Cl ion, whose mitogenetic radiation takes place in the form of a sensitized fluorescence [4]. The spectrum of sensitized fluorescence of the Cl ion was studied in 1939 between limits of 1900-2500 A [5], and hence in this range the chloride lines were excluded by simple comparison. However, on determining the spectrum of acetylcholine, mitogenetic effect was also found in the range 2600-2700 A; for this reason in order to exclude the possibility that this was due to chloride we performed an additional series of experiments to study the sensitized fluorescence of a solution of NaCl in the range 2500-3200 A.

The results of this series of experiments showed no effect on the lines in the range 2600-2700 A from sensitized fluorescence of Cl and Na ions. Consequently the effect on this area, which was obtained in the experiments to study the radiation from acetylcholine chloride solution, occurs as the result of the acetylcholine molecule. The spectrum of acetylcholine thus obtained included three lines 5 A wide (1945-1950 A, 2335-2340 A, 2375-2380 A) and one line 10 A wide (2620-2630 A) (see figure).**

* Fluess spectrograph. Transliterated.

** This refers, of course, to maxima of intensity of radiation in given areas of the spectrum, i.e., to the so-called half-breadth of the lines.



Mitogenetic spectrum of acetylcholine.

The spectral lines obtained were checked by the method of sensitized fluorescence of an acetylcholine solution of a concentration $0.5 \cdot 10^{-2}$. As a control, in addition to the spectral lines of acetylcholine, adjacent areas of the spectrum, 5 A wide, situated in the long and short wave sides of the main line were checked. The results of the experiments showed the presence of mitogenetic effect in the region of the main lines and its absence from the adjacent areas at the sides. This coincidence of the spectra of spontaneous radiation and of sensitized fluorescence of acetylcholine was confirmation of the correctness and accuracy of the spectrum obtained.

TABLE 1

Test of the Acetylcholine Lines in the Resting Muscle*

Wavelength in A	Intensity of acetylcholine line in % exposure in sec			
	20	30	40	60
1940—1945	—	—13	9	—9
1945—1950	—5	0	5	9
1950—1955	—	—6	6	—4
2330—2335	—	0	—	0
2335—2340	1	—4	—6	4
2340—2345	—	—15	3	10
2370—2375	—	4	11	20
2375—2380	10	8	2	3
2380—2385	—	—2	6	6
2615—2620	—	—7	8	—3
2620—2630	—11	—4	—2	5
2630—2635	—	10	—7	—6

* In this and subsequent tables the figures show the mean values of several experiments. The variation of the figures between limits of ± 15% gives the margins of error of the statistical method of biodetection.

The second part of the work consisted of the detection of acetylcholine in stimulated muscle in vivo by means of the standard spectrum obtained. The experiments were performed on the upper third of the gastrocnemius muscle (an area 4 × 3 mm) from which the skin had been removed. The frog was pinned sparingly to a board and wrapped over with a damp cloth. In all the experiments adjacent spectral areas to the main lines, 5 A in width, on both the long and short wave sides were checked.

Experiments on resting muscles with exposures of 20, 30, 40 and 60 sec showed absence of radiation both at the main lines and in the areas at the sides. In experiments on resting muscles their spontaneous radiation was tested at the end, giving a maximum which was concentrated in the region of 2200-2300 A, at an optimal exposure of the order of 8-10 sec. The spontaneous radiation acted as a criterion of the satisfactory physiological condition of the muscle, for only in such a case did we consider the results of the main experiment to be valid (Table 1).

In the experiments to detect acetylcholine in the stimulated muscle two forms of stimulation were used. In the first experiments, stimulation of the sciatic nerve was effected by electric impulses from a stimulator of the GRAKh-1 system. The physical characteristics of stimulation were: form of impulse — square, duration of impulse — 5 msec, frequency — 25 hertz. At threshold amplitude (varying in different specimens from 0.7 to 1.7 v) weak, rhythmical contractions of the gastrocnemius muscle were obtained. Rest periods of 1-2 min were given between exposures, and after six stimulations the frogs were replaced. Exposures of 5 and 8 sec were effective.

In the second series of experiments a more physiological tactile stimulus was used on the contralateral limb, by means of a revolving disc operated by an electric motor. The edge of the disc which performed the stimulation was bent at right angles to its surface; in consequence of the graded reduction in the radius of the disc, which gave the edge of the disc a graded spiral shape, this device caused continuous and successive stimulation of the whole of the skin of the limb from the foot to the upper third of the thigh. With correct selection of the threshold of stimulation, regulated by altering the speed of revolution of the disc, a weak interrupted tetany of the whole musculature of the contralateral limb was obtained. Duration of exposures — 8 and 12 sec.

TABLE 2

Test of the Acetylcholine Lines in the Muscle During Electrical Stimulation of the Nerve*

Wavelength in A	Intensity of the acetylcholine lines in %	
	exposure in sec	
	5	8
1940—1945	4	—9
1945—1950	36	62
1950—1955	—2	0
2330—2335	—10	—2
2335—2340	29	33
2340—2345	—2	14
2370—2375	8	8
2375—2380	60	75
2380—2385	4	—5
2615—2620	0	7
2620—2630	8	26
2630—2635	0	—

* The difference in the intensity of individual acetylcholine lines is not surprising in view of the different spectral data.

TABLE 3

Test of the Acetylcholine Lines in the Muscle During Tactical Stimulation of the Contralateral Limb

Wavelength in A	Intensity of the acetylcholine lines in %	
	exposure in sec	
	8	12
1940—1945	—4	—8
1945—1950	54	10
1950—1955	9	9
2330—2335	—11	0
2335—2340	10	29
2340—2345	7	7
2370—2375	4	4
2375—2380	40	—14
2380—2385	2	—2
2615—2620	8	—8
2620—2630	—2	20
2630—2635	—	—2

The results of both series of experiments showed clearly a marked mitogenetic effect in all 4 acetylcholine lines and no effect in series of lateral areas of the spectrum 5 A in width (Tables 2 and 3).

The results obtained suggest that during stimulation acetylcholine appears in the muscle in the free state. The immediate purpose of the investigation — the possibility of detection of acetylcholine in vivo by the method of mitogenetic spectral analysis — was thus fulfilled.

The view that the acetylcholine detected spectrally was identical with that detected during perfusion of a stimulated muscle was most convincing. This view does not touch the question whether this acetylcholine is the mediator secreted by the nerve endings, according to the classic neurohumoral theory (O. Levi, Dale, A. V. Kibiakov) or whether it appears in the muscle as a result of leakage according to the concept of Nachmansohn [9]; the latter attributes to acetylcholine the leading role in the generation of action currents and considers that there is no intrinsic difference in carrying out stimulation through the axon or the synapse. The possibility cannot be entirely excluded also that the acetylcholine found in large quantity in nerve plexi and endings during stimulation may also be picked up spectrally, which can be judged indirectly by the high concentration of cholinesterase in these structures.*

SUMMARY

Experimental data demonstrate the possibility of detection of acetylcholine with the aid of mitogenetic spectral analysis in the frog's muscle in vivo.

The spectrum of acetylcholine may be easily revealed during the stimulation of the gastrocnemius. There are no characteristic spectral lines when the muscle is at rest.

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PHARMACOLOGY

THE EFFECT OF PAPAVERINE AND DIBAZOL* ON THE RATE OF THE CORONARY CIRCULATION

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The drugs used in the treatment of angina pectoris exert different effects on the coronary vessels. In this respect their comparative experimental evaluation is of great interest. We began the study of the drugs used in the treatment of coronary insufficiency with an investigation of papaverine and dibazol.

The effect of papaverine on the coronary vessels was studied experimentally at first on the isolated heart [5, 7, 10], and later on the heart-lung preparation [8] and on the heart in situ [3, 4, 9]. However, no detailed investigation of the effect of papaverine on the coronary vessels of the intact animals has yet been made.

Despite the clinical use of dibazol there is insufficient experimental confirmation of its effect on the coronary vessels. The references in the literature to this problem are confined to the work of D. S. Paskov [2], who showed that dibazol causes slight dilation of the lumens of the coronary vessels of the isolated human heart. Generally speaking no investigation of the effect of dibazol on the coronary vessels has been carried out in the intact animal. Similarly no study has been made of the combined effect of papaverine and dibazol on the rate of the venous blood flow. Nevertheless a combination of the two drugs is often used in clinical practice.

EXPERIMENTAL METHOD

In this work we used a method which permitted determination of the rate of flow of a volume of blood from the coronary sinus. The experiments were performed on cats under nembutal anesthesia (40 mg/kg). With the application of artificial respiration to the animals, the thorax was opened and a polythene catheter introduced into the coronary sinus through the right auricle, after which heparin was injected (1500 units/kg body weight of the animal). The blood from the coronary sinus passed along the polythene catheter into a measuring tube and caused a small float to rise, the movement of which was recorded mechanically on the smoked drum of a kymograph. The volume of blood flowing from the coronary sinus in 15 min was measured periodically. A detailed account of the method was given in the paper by N. V. Kaverina [1].

The size of the blood flow was estimated in ml/min per 100 g weight of the heart. Normally the mean volume of blood flowing from the coronary sinus of the cat amounted to 43 ml/min per 100 g weight of the heart (results of 35 experiments) with a mean square deviation of 13.8. This value of the rate of the blood flow in the heart corresponds approximately to the figures obtained by Gregg in experiments on dogs.

The blood pressure in the carotid artery was recorded. The tested drugs were injected intravenously.

* Dibazol is 2-benzyl-benzimidazol hydrochloride – Publisher's note.

EXPERIMENTAL RESULTS

Experiments with Papaverine

The character of the effect of papaverine on the rate of the coronary blood flow may be judged from Fig. 1: in an effective dose (2 mg/kg) papaverine causes a marked increase in the rate of the blood flow from the coronary sinus.

TABLE 1

The Effect of Papaverine and Dibazol on the Rate of the Coronary Blood Flow

Drug	Dose in mg/kg	No. of expts.	Maximum increase in rate of coronary blood flow in %		Duration of action in minutes	
			mean values	limiting values	mean values	limiting values
Papaverine	0.5	10	28	10 & 70	10	3 & 15
	1	6	44	26 & 55	12	3 & 40
	2	6	72	38 & 116	45	20& 55
	4	1	114	—	1 hr 30 min	—
Dibazol	3	5	18	10& 24	Duration of action was 2-3 min irrespective of dose. It reached 10 min in only one experiment.	
	5	6	30	15& 43		
	10	5	39	33& 60		
	20	1	10	—		

The summarized results of the series of experiments with papaverine are shown in Table 1. With increase in the dose of papaverine the strength and duration of its action increased. In a dose of 0.5 mg/kg papaverine causes maximum increase in the rate of the coronary blood flow by 28% for 10 min; in a dose of 4 mg/kg, in spite of a sharp fall in blood pressure, it increases the rate of the blood flow by 114% and its action lasts 1.5 hrs.

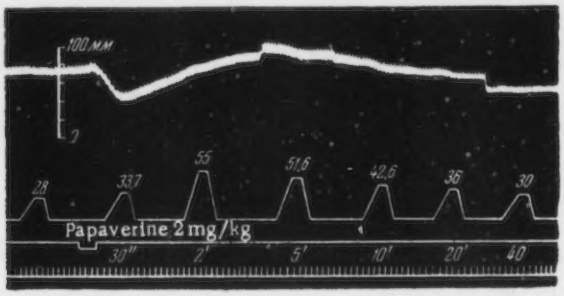

Fig. 1. The effect of papaverine on the rate of the blood flow from the coronary sinus. Interpretation of the curves (from above downwards): arterial pressure, blood flow from the coronary sinus, stimulation marker, time marker (5 sec). The figures indicate rate of flow of blood in ml/min per 100 g weight of the heart. The figures below the line of the time marker indicate the length of time after the injection of the drug that determination of the rate of the blood flow was made.

The characteristic feature of the reaction of the coronary vessels to papaverine in different animals is its great variability. In a dose of 0.5 mg/kg papaverine causes an increase of 10-70% in the rate of the coronary

blood flow. In some animals even greater doses of papaverine do not increase the rate of the coronary blood flow.

With repeated injections of papaverine an increased reaction of the coronary vessels to each successive injection was not observed.

In order to ascertain the role of the cholinergic and adrenergic innervation in the vasodilator effect of papaverine, experiments were performed using atropine and dihydroergotamine. After administration of atropine in a dose of 1 mg/kg the increase in the rate of the coronary blood flow in response to papaverine was approximately the same as before the atropine was given. The reaction of the coronary vessels to papaverine was also unchanged after the injection of 0.5 mg/kg of dihydroergotamine. Thus the effect of papaverine on the rate of the coronary blood flow is evidently independent of the action of this drug on the innervation of the vessels of the heart.

Experiments with Dibazol

Figure 2 represents an experiment in which the effect of dibazol on the rate of the coronary blood flow was most marked. The results of the effect of dibazol on the rate of the coronary blood flow are summarized in Table 1. From this table, and from Fig. 2 it can be seen that the rate of the coronary blood flow is slightly increased by dibazol for only a very short period of time. In a dose of 20 mg/kg dibazol had less effect than in a dose of 10 mg/kg, on account of the sharp fall in the blood pressure.

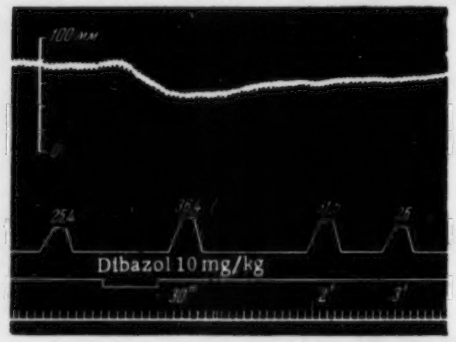

Fig. 2. The effect of dibazol on the rate of blood flow from the coronary sinus. Interpretation as in Fig. 1.

With repeated administration the reaction of the coronary vessels to dibazol was increased. In one experiment dibazol, injected in a dose of 1 mg/kg half an hour after the injection of the same drug in a dose of 15 mg/kg, caused an increase in the rate of the coronary blood flow of 54%, while at the beginning of the experiment a dose of 1 mg/kg was below the threshold level.

Just as in the experiments with papaverine, the preliminary administration of atropine and dihydroergotamine caused no essential changes in the reaction of the coronary vessels to dibazol.

Experiments with Combined Administration of Papaverine and Dibazol

The injection of papaverine immediately after dibazol increases the rate of the coronary blood flow to a greater degree than its use alone. The results of this series of experiments are shown in Table 2. The papaverine was usually injected from 10-15 min after the injection of dibazol. At this time the increase in the rate of coro-

TABLE 2

The Effect of Papaverine on the Rate of the Coronary Circulation Before and After Injection of Dibazol

Expt. no.	Effect of papaverine (0.5 mg/kg) on rate of coronary blood flow in %		Dose of dibazol in mg/kg	Effect of papaverine (0.5 mg/kg) on rate of coronary circulation after injection of dibazol	
	maximum increase in rate of coronary blood flow in %	duration of action in min		maximum increase in rate of coronary blood flow in %	duration of action in min
1	50	20	10	60	60
2	20	5	10	80	10
3	25	10	20	65	10
4	35	10	10	60	20
5	28	10	10	38	20

nary blood flow due to dibazol had completely disappeared. In 2 experiments papaverine was injected 10 min and also one hour after the injection of dibazol. In the latter case the increase in the reaction of the coronary vessels to papaverine was not observed.

With combined administration of papaverine and dibazol an enhanced effect of the drugs on the rate of the coronary blood flow is observed. In one of these experiments in which this effect was most pronounced, papaverine in a dose of 0.5 mg/kg and dibazol in a dose of 2.5 mg/kg when injected simultaneously caused an increase in the rate of the blood flow of 35% which lasted 30 min.

Papaverine alone in the same dose increased the rate of the coronary blood flow by 10% for a period of 10 min, while dibazol increased the rate of the coronary blood flow by 10% for 2 min.

SUMMARY

The effect of papaverine and dibazol on the coronary blood flow was studied in short-term experiments on cats, anesthetized with nembutal in condition of artificial respiration. A method which allowed determination of the volume velocity of blood outflow from the coronary sinus was employed. Papaverine provokes a pronounced increase of the coronary blood flow. Dibazol on the other hand, causes only insignificant and temporary increase. When papaverine was administered after dibazol its effect on the coronary blood vessels was enhanced. Thus, combined administration of papaverine and dibazol reinforces their effect.

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MICROBIOLOGY AND IMMUNITY

THE RELATION OF THE SITE OF ACTION OF THE ANTIGEN IN THE BODY AND THE SITE OF ANTIBODY FORMATION

(In Experimental Immunization with Tetanus Toxoid)

COMMUNICATION III. A COMPARISON OF THE VALUE OF INTRAMUSCULAR AND INTRAVENOUS INJECTION OF TOXOID AND THE IMPORTANCE IN IMMUNIZATION OF ANTIGEN CIRCULATING IN THE BLOOD

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It was shown in a previous paper [5] that the intramuscular injection of toxoid has no advantage over intravenous injection from the point of view of the concentration and duration of the presence of antigen in the blood, although it is considerably more effective in immunization. From these facts there arose the hypothesis that the basic sites of immunizing activity after intramuscular injection of toxoid are the organs and tissues which take up the antigen before it enters the blood stream, i. e., the regional lymphatic glands or the muscles. In order to provide grounds for this hypothesis it was necessary to compare the effectiveness of the intramuscular method of immunization with the effectiveness of the intravenous injection of antigen according to a scheme would imitate the gradual absorption of antigen from the muscle and also prolong its circulation to a known degree. In working out this scheme we made use of previously obtained findings [5], according to which the duration of absorption of toxoid from the muscle does not exceed 3-5 days, and moreover the greater part of the antigen is taken up during the first 24 hrs.

EXPERIMENTAL METHOD AND RESULTS

For the experiments we selected 10 rabbits of the chinchilla family, mainly males; their average weight was 2.3 kg. During the course of 4 days they were injected intravenously 12 times in the auricular vein with tetanus toxoid (series No. 336-4, obtained from the N. F. Gamaleia Institute of Epidemiology and Microbiology of the Academy of Medical Sciences, USSR), diluted with 10 times its volume of physiological saline. The total dose of toxoid injected into each rabbit was 0.5 ml in a volume of 5 ml, and the individual dose varied from 0.02 to 0.05 ml in a corresponding volume. The intervals between injections gradually increased from 2 to 24 hrs.

Another group of animals (7 rabbits of the same sex and weight) were given a single injection of 0.5 ml of undiluted toxoid into the rectus femoris muscle. Part of the animals (3 rabbits) received in addition intravenous injections of physiological saline according to the same scheme followed in immunizing the animals of the first group.

Investigation of the toxoid content of the blood serum of the experimental animals by a modification of Becher's [4] method showed that the antigen circulated for a longer time in the blood of the first group of rabbits than after intramuscular immunization. Two months later all the animals were revaccinated intravenously with a single injection of 0.5 ml of toxoid.

After the primary immunization, and also after revaccination systematic estimations were made of the antitoxin content of the blood sera of the animals by the usual titration in mice. The results obtained are shown in Fig. 1.

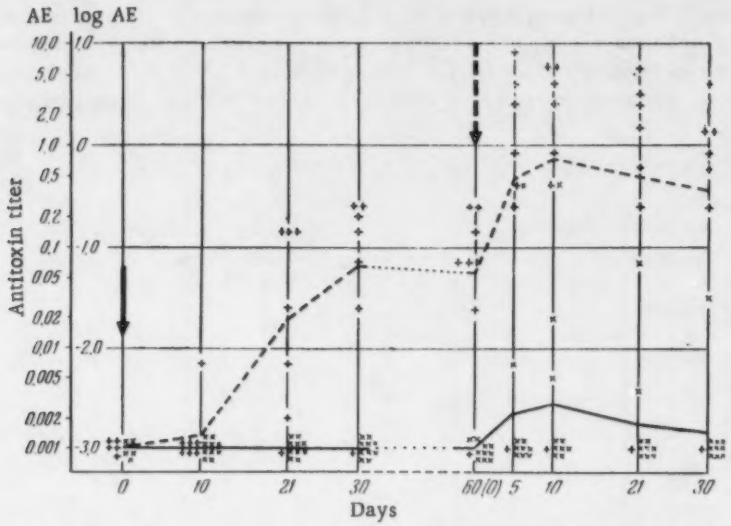

Fig. 1. Comparative effectiveness of fractional intravenous and single intramuscular immunization. Conventional signs: ↓) primary immunization; x) individual antitoxin titers after fractional intravenous injection of antigen (primary immunization); ——) their geometric mean; +) individual antitoxin titers after intramuscular injection of antigen (primary immunization); ---) their geometric mean; ↓) revaccination (intravenously). Here, and in Fig. 2, antitoxin titers below 0.002 antitoxin units (threshold of sensitivity of the method) are conventionally indicated as being equal to 0.001 units. AE - Antitoxin units.

As can be seen in Fig. 1, fractional intravenous immunization did not cause antibody formation in one of the 10 rabbits, and the majority of the animals (7 out of 10) remained indifferent also to the revaccination injection of antigen.* In the majority of rabbits (6 out of 7) immunized intramuscularly, however, antibody formation was observed; subsequent intravenous injection of antigen brought about in these animals the characteristic rapid rise in the antitoxin titer associated with revaccination.

Thus injection of toxoid directly into the blood stream caused the formation of antibodies only in the presence of previous immunizational reorganization of the body as a result of intramuscular immunization, but could not as a rule bring about this reorganization itself despite the prolonged circulation of the antigen in the blood. A similar enhancement of the intravenous immunization effectiveness as a result of previous injection of antigen into the tissues is also known in the case of diphtheria toxoid [8].

However, the possibility was not excluded that during intramuscular injection an altered antigen is absorbed into the blood, more active so far as immunization is concerned. Although in investigation of the revaccinating

* In assessing the possible causes of the rise in antitoxin titers observed in certain rabbits after revaccination, one has to take into consideration the unavoidable leakage of small quantities of antigen into the tissues in the process of repeated intravenous injections. As shown later, injection into the tissues of a subthreshold dose of antigen in conjunction with intravenous injection of toxoid causes a satisfactory immunizing effect. Additional data in support of such a view will appear in one of the later reports.

activity of sera from blood taken after intramuscular or intravenous immunization were not essentially different [5], this possibility mentioned must be checked once more by another method. For this purpose we examined the immunizing effect of intravenous injection of toxoid previously incubated in muscle in vivo.

We injected 11 donor rabbits of both sexes with 1.5 ml of toxoid into the right and left rectus femoris muscles. These muscles were excised after an interval of time from a few minutes to 24 hours, and then were ground up for 5 min in 3 times their volume of physiological saline in a Waring type blender (made in the experimental factory of the Academy of Medical Sciences, USSR). The ground-up mass was centrifuged for 7-8 min at 2500 rev/min; the sediment was discarded. All the operations were performed as quickly as possible, with sterile precautions, and in the cold; penicillin was added to the prepared extracts in a dose of 3000 IU per 1 ml. The extracts were injected intravenously into 5 recepient rabbits according to the 12 injection scheme described above, and the schedule of injections of the extracts corresponded in duration to the incubation of the antigen in the muscle of the donor. A control group of recipients (3 rabbits) was immunized intramuscularly with 0.5 ml of

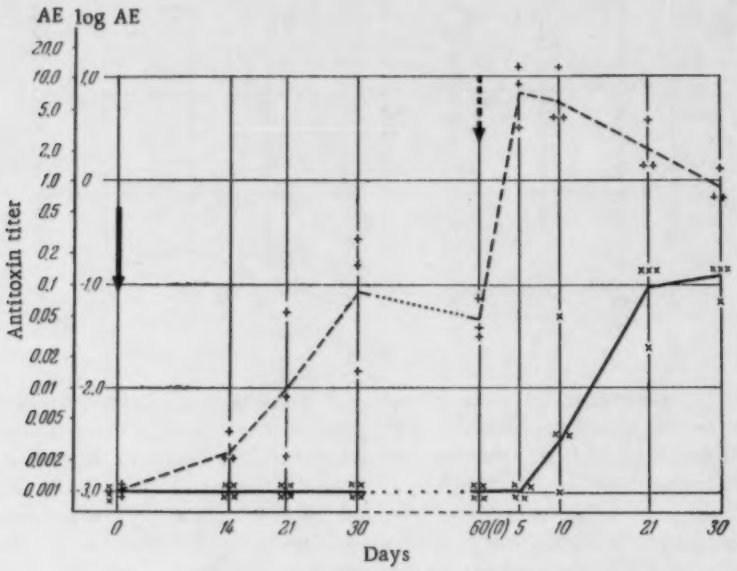

Fig. 2. Effectiveness of intravenous immunization with toxoid incubated in muscle. Conventional signs: ↓) primary immunization; x) individual antitoxin titers of the experimental group of rabbits; ——) their geometrical mean; +) individual antitoxin titers of control animals immunized intramuscularly with native antigen; ---) their geometrical mean; ↓) revaccination (intramuscularly). AE- Antitoxin units.

native toxoid; in order to equalize the experimental conditions this group of animals received intravenously a muscle extract from an unimmunized donor. The total dose of extract amounted to 45 ml for each rabbit, the individual doses from 2 to 5 ml. Injection of the extract as a rule caused no apparent reactions (rarely slight shortness of breath). The only exception was one rabbit of the experimental group which died 10-15 min after the 10th injection. At necropsy extensive recent hemorrhages were found beneath the endocardium of the ventricles (particularly the right) and widespread areas of hepatization in the lungs.

Judging by the antitoxin-combining activity of the extracts, each recipient in the experimental group received a dose of antigen equivalent to 0.6± 0.3 ml of native toxoid. In the blood sera of the recipients of the first group the antigen content either was approximately equal to (24 hrs after immunization) or greater than (3 days afterwards) the corresponding values in the control group.

After 60 days all the recipient rabbits were revaccinated intramuscularly with 0.5 ml of toxoid. Their blood antitoxin content was systematically investigated (after both primary immunization and revaccination). The results obtained are shown in Fig. 2.

As can be seen in Fig. 2, rabbits immunized intramuscularly with native toxoid showed the typical effect of primary immunization with maximum titers on the 30th day, and after the subsequent injection of antigen – a sharp revaccination rise in the titers with a maximum on the 5th-10th day. In those rabbits, however, which were immunized with intravenous toxoid previously incubated in muscle, antitoxin production did not take place, and subsequent intramuscular injection of antigen caused only the ordinary primary immunization effect. Thus the antigen circulating in the blood stream in these experiments had no power to cause either antibody formation or to increase the reactivity of the animal to subsequent injection of antigen.

It does not follow from this, however, that toxoid circulating in the blood is in general unable to play any part in the formation of the primary immunization reaction. This is shown convincingly by the results of simultaneous intravenous and intramuscular immunization of rabbits. The first group of animals were given daily injections for four days of tetanus toxoid, diluted 5 times with physiological saline, into the marginal vein of the ear. The total dose of toxoid for each rabbit was 0.5 ml in a volume of 2.5 ml; the individual doses were gradually reduced from 0.16 to 0.06 ml. The second group of rabbits were given a single injection into the rectus femoris muscle of undiluted toxoid in a dose of 0.5 ml. The third group of animals received a single intramuscular injection of 0.15 ml of toxoid diluted to 0.5 ml. Finally, the fourth group of animals also received 0.15 ml of toxoid diluted to 0.5 ml intramuscularly and in addition 0.35 ml of toxoid intravenously (the first injection was given at the same time as the intramuscular injection) by a scheme similar to that used in the immunization of the first group of rabbits. To make the experimental conditions equal, animals immunized intramuscularly were given additional intravenous injections of physiological saline, and vice versa. After 3 weeks, as shown by investigation of the antitoxin titers (see table), the animals of the second and fourth groups showed a well-marked immunization reaction, those of the third group a very weak reaction and those of the first group no reaction at all. It follows from these findings that although by itself the toxoid circulating in the blood (in accordance with the findings of the preceding series of experiments) has no power to cause the formation of antibodies, an increase in its concentration intensifies the effect of intramuscular immunization with a subthreshold dose.

The Immunizing Effectiveness of Fractional Intravenous and Single Intramuscular Injection of Antigen, and of Their Combined Administration

Group of animals	Scheme of immunization	Number of rabbits	Immunizing effect on the 21st day (M± 2m)	
			% of animals with antitoxin in the blood (≥ 0.002 antitoxin units)	average titers in antitoxin units
First	0.5 ml of toxoid intravenously (fractional)	5	0^{+33}_{-0}	< 0.002
Second	0.5 ml of toxoid intramuscularly	12	83^{+17}_{-23}	0.120± 0.063
Third	0.15 ml of toxoid intramuscularly	14	36± 27	$0.016^{+0.022}_{-0.016}$
Fourth	0.15 ml of toxoid intramuscularly + 0.35 ml of toxoid intravenously (fractional)	14	86^{+14}_{-18}	0.111± 0.087

Thus the high effectiveness of the intramuscular method of immunization compared with the intravenous cannot be explained either by the longer circulation of antigen in the blood nor by any form of activation of the antigen in the muscle tissue. Toxoid injected into the blood stream in conditions which imitate its natural absorption was found as a rule to be incapable of causing any alteration in the immunological reactivity of the animal. The decisive factor in bringing about the primary immunization reorganization of the body must therefore be regarded as the action of the antigen on those organs and tissues penetrated by the toxoid before it enters the general circulation. In intramuscular immunization these organs may be the muscles or regional lymphatic glands. According to some reports [14], tetanus toxin, like other proteins with a molecular weight over 20,000, enters the circulation mainly, or even entirely, by the lymphatic route. Evidently the same must occur with absorption of tetanus toxoid, whose molecular weight is not less than 68,000 [12]. The low power of penetration of the blood capillaries by toxoid is shown by its prolonged deposition in the muscle and its circulation in the blood [5].

Analyzing the possible causes of the narrow localization of the initial phases of the immunization activity, it must be pointed out that the higher effectiveness of immunization by injection directly into the tissues compared with the intravenous method is also characteristic of other molecularly dispersed antigens [1, 3, 6, 7, 8, 11, 15], irrespective of their specific properties. However, in the case of corpuscular antigens (erythrocytes, killed cultures of bacteria, and so on) a number of writers [1, 2, 10, 16] found the converse to be true. There are also reports [7] of the increased effectiveness of intravenous injection of antigen if it is adsorbed on alumogel. It seems likely that the low immunizing activity of a molecularly dispersed antigen circulating in the blood stream is due to a considerable extent to the low concentration of antigen compared with that arising in the tissues and lymphatic system after the intramuscular method of injection. The correctness of this attitude to the question is confirmed by the findings of Glenny and his co-workers [9] who observed a considerable reduction in the immunizing effect by dilution of diphtheria antigen injected intramuscularly with physiological saline. On the basis of the general physiological ideas of a threshold of excitability of a biological structure, it might be thought that the reorganization of the body in primary immunization cannot be brought about by the taking up of solitary molecules of antigen even by a large number of cells; it demands, on the contrary, the action of a sufficiently massive dose of antigen even though the number of appropriate functional units (cells forming antibody, or nerve endings) is restricted.* It is clear that in the case of immunization with corpuscular vaccines this condition is satisfied also during their intravenous injection. In the course of the immunizational reorganization of the body the sensitivity to the antigen rises sharply; thus in our experiments [5] the injection of toxoid diluted even 1000 times had a significant revaccinal effect. This makes clear the effectiveness of intravenous revaccination mentioned above. These ideas which we have discussed may be of value in the future study of the mode of action of the so-called adjuvants.

SUMMARY

The content of toxoid was investigated in the blood serum in certain methods of intravenous and intramuscular immunization. The period of circulation of the antigen in the blood as well as its possible qualitative changes were examined. The data obtained permit the belief that toxoid circulating in the blood is of secondary significance in the primary immunizational reactions of the organism. The effect of antigen on the regional lymph nodes or on the tissues at the site of injection should be considered as a decisive factor. The question of dependence of the place of action of the antigen on its physicochemical structure is discussed.

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* As shown by the investigations of Leduck et al. [13], there is a marked disproportion between the large number of cells taking up antigen and the small number of cells producing antibody.

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THE USE OF THE REACTION OF INCREASE OF PHAGE TITER IN THE DETECTION OF FLEXNER DYSENTERY BACTERIA IN INFECTED RABBITS

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The spread of dysentery bacteria throughout the human body is of considerable interest from the point of view of solving problems of the pathogenesis and also of specific prophylaxis of dysentery. The views expressed in the literature on the spread of dysentery microorganisms in the body are based on bacteriological detection of the agent in the blood or in organs of persons dying from dysentery [3, 4], and on bacteriological examinations of the blood and bile during life (Denner, Kulesha, Besredka et al.).

In recent years series of experiments have been performed to study the pathogenesis by means of radioactive isotopes. V. I. Ivanov and his co-workers [2] describe their findings of the distribution in animals of antigens of typhoid bacteria containing radioactive phosphorus P^{32}. V. L. Troitskii and his co-workers [8], using Flexner-Sonne dysentery vaccine labeled with radiophosphorus, demonstrated rapid penetration of microorganisms into the blood stream after infection of rabbits per os. The results so far obtained suggest that other methods of indication of dysentery microorganisms in the body are necessary in order to elucidate the problems of pathogenesis and immunity in dysentery.

In the present paper we describe an experimental study of the possibility of using the reaction of increase of phage titer (the method of V. D. Timakov and D. M. Gol'dfarb) to detect dysentery microorganisms in infected rabbits. This reaction was widely tested by these authors for diagnostic purposes in detecting dysentery bacteria in the stools of patients and carriers, and also in various environmental objects (in water, and washings from the hands and various utensils).

EXPERIMENTAL METHOD

The principle of the reaction is based on the increase in the titer of highly sensitive indicator phage during its interaction with dysentery bacteria contained in the material for examination. It enables these microorganisms to be found in a short time (10-20 hrs) in very low concentrations. The experiment was performed on 53 rabbits, divided into 4 groups. The animals were infected with a mixture of dysentery strains of Flexner a, b and c types, whose virulence, expressed LD_{100}, was equal to 500 million bacterial bodies per 1 ml. For infection we used washings in physiological saline of 24-hr cultures with an optical standard of 1 and $4 \cdot 10^9$ bacterial bodies per 1 ml.

The animals were infected by groups – intravenously, subcutaneously and orally, mainly with the $1 \cdot 10^9$ suspension. In the group of animals infected orally the experiment was repeated with the $4 \cdot 10^9$ suspension (4th group). In order to confirm the reliability of the results in each group there were control rabbits. The infected animals were killed at various intervals – 1, 3, 6, 24, 48 and 72 hrs and 5, 15 and 30 days after administration of the infective material. The following organs were investigated: liver, spleen, kidney, lungs, lymphatic glands, mucous membrane of the small and large intestine, ascending and descending colon bone marrow and brain.

In the specimens obtained the presence of dysentery bacteria was determined by the reaction of increase of phage titer* and by a bacteriological method. For the RIP we used indicator phage obtained from the Central Institute of Epidemiology and Microbiology.

The RIP was estimated by the increase in the number of patches (sterile areas). An increase in the number of patches of 3-5 times that of the control phage in a degree of 10^{-8} was regarded as a + reaction, from 5-7 times as ++, from 7-10 times as +++ and over 10 times as ++++.

EXPERIMENTAL RESULTS

Altogether we performed 90 analyses of organs from rabbits infected intravenously (9 animals, excluding controls). In the experiments on organs of animals examined 1 and 3 hrs after infection the results of the RIP and bacteriological investigations agreed. It was found that at 1 hr after infection a culture was found by both methods in the liver, spleen, bone marrow, lung, lymphatic glands and kidney; after 3 hrs, in addition to the organs just mentioned it was found in the descending colon and the brain. After 6 hrs we obtained a positive result in all the organs examined except the lymphatic glands and the ascending and descending colon, where bacteria were discovered only by means of the RIP, cultures giving negative results (Table 1).

The changes in the discoveries of the microorganisms in the subsequent hours and days show that until 3 days the almost complete dissemination of microorganisms in the examined organs persists; later they begin to disappear and on the 30th day after infection they can no longer be detected. In some cases, as seen from Table 1, the agent could be found only by means of the RIP, in particular in the mucosa of the large intestine, from 3 hrs after infection until the 15th day. The quantitative changes in the microorganisms in the intestinal mucosa are well reflected by the intensity of the RIP, which weakens sharply at 48 hrs.

The results of this series of experiments showed that in 62 positive analyses the results of the RIP and the bacteriological method agreed in 52 cases (83.8%); 10 analyses, or 16%, gave a positive RIP along with a negative bacteriological examination. The use of the RIP along with the bacteriological method in this case showed that after infection of rabbits by the intravenous method the agent could be detected in the mucosa of the colon only 3 hrs after infection, and persisted stubbornly in all parts of the intestine for 5 days, while it could be observed in the ascending colon on the 15th day after infection.

Nine animals were infected subcutaneously (1 ml of $1 \cdot 10^9$ bacterial suspension). The material was injected into the middle of the abdomen. The rabbits were killed and examined on the same days and hours. Microorganisms were found by both methods after 48 and 72 hours (Table 2) in the ascending colon. On the 5th day they were found only by means of the RIP in the following organs: lymphatic glands, small intestine, ascending and descending colon, bone marrow. In subsequent days (15th and 30th days) all the analyses were negative (see Table 2).

The subcutaneous infection experiment also demonstrated the selective power of the dysentery organisms to become localized in the mucosa of the small and large intestine. In addition the greater sensitivity of the RIP in comparison with the bacteriological method of investigation was established.

The 3rd and 4th groups, each of 9 rabbits, were infected orally with 1 and $4 \cdot 10^9$ suspensions of bacteria. In the 3rd group, positive bacteriological examination and RIP results were obtained with lung tissue 1 and 3 hrs after infection. In addition a positive culture and negative RIP were found in the bone marrow after 1 and 48 hrs. A positive RIP alone was also found in the lymphatic glands and the mucosa of the descending colon after 48 hrs. At this time the positive results by both methods were in agreement for cultures isolated from the mucosa of the descending colon.

* Abbreviated RIP in text.

TABLE 1

Isolation of Dysentery Microorganisms After Intravenous Infection with a $1 \cdot 10^9$ Suspension

Organ	Time of necropsy of animals																	
	1 hr		3 hrs		6 hrs		24 hrs		48 hrs		72 hrs		5 days		15 days		30 days	
	RIP	b.m.	RIP	b.m.	RIP	b.m.	RIP	b.m.	RIP	b.m.	RIP	b.m.	RIP	b.m.	RIP	b.m.	RIP	b.m.
Liver	+	+	++	+	++++	+	++++	−	++	−	+	+	++++	−	−	−	−	−
Spleen	++++	+	++	+	++++	+	++++	+	++	+	++	+	−	−	−	−	−	−
Kidney	++++	+	++	+	+++	+	++	+	++	+	+	+	+	−	−	−	−	−
Lung	+	+	+	+	++	+	+++	+	+	+	+	+	−	−	−	−	−	−
Lymphatic gland	++++	+	++++	+	++++	−	++++	+	+	+	+	+	+	+	−	−	−	−
Small intestine	−	−	−	−	++++	+	++++	+	+	+	+	+	+	+	−	−	−	−
Ascending colon	−	−	−	−	++++	−	++++	+	+	+	+	+	++	+	+	+	−	−
Descending colon	−	−	++++	+	++++	−	++++	−	+	+	+	+	++	+	−	−	−	−
Brain	−	−	+	+	++++	+	−	−	++	+	++	+	−	−	−	−	−	−
Bone marrow	++++	+	++	+	++++	+	+	−	+	+	+	+	+	−	−	−	−	−

Notes: 1) b. m. – bacteriological method (in this and in the subsequent tables); 2) RIP and the bacteriological examination are taken as 1 analysis.

TABLE 2

Isolation of Dysentery Organisms After Subcutaneous Infection of Rabbits with a $1 \cdot 10^9$ Suspension

Organs	Time of necropsy of animals																	
	1 hr		3 hrs		6 hrs		24 hrs		48 hrs		72 hrs		5 days		15 days		30 days	
	RIP	b.m.	RIP	b.m.	RIP	b.m.	RIP	b.m.	RIP	b.m.	RIP	b.m.	RIP	b.m.	RIP	b.m.	RIP	b.m.
Liver	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Spleen	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Kidney	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Lung	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Lymphatic gland	–	–	–	–	–	–	–	–	–	–	–	–	++	–	–	–	–	–
Small intestine	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–
Ascending colon	–	–	–	–	–	–	–	–	++++	+	+	+	++	–	–	–	–	–
Descending colon	–	–	–	–	–	–	–	–	–	–	–	–	++	–	–	–	–	–
Brain	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Bone marrow	–	–	–	–	–	–	–	–	–	–	–	–	++	–	–	–	–	–

TABLE 3

Isolation of Dysentery Organisms After Oral Infection with a $4 \cdot 10^9$ Suspension

Organs	Time of necropsy of animals																	
	1 hr		3 hrs		6 hrs		24 hrs		48 hrs		72 hrs		5 days		15 days		30 days	
	RIP	b.m.	RIP	b.m.	RIP	b.m.	RIP	b.m.	RIP	b.m.	RIP	b.m.	RIP	b.m.	RIP	b.m.	RIP	b.m.
Liver	+++	+	−	−	+++	−	+++	−	−	−	+	−	−	−	−	−	−	−
Spleen	++++	+	+++	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Kidney	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Lung	++++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Lymphatic gland	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
Small intestine	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Ascending colon	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−
Descending colon	−	−	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−
Brain	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Bone marrow	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−

The oral infection experiments were repeated with a thicker ($4 \cdot 10^9$ bacterial bodies per 1 ml) bacterial suspension in order to confirm the possibility of hematogenous spread of dysentery microorganisms, which was suggested by their discovery in the lungs and other organs in the first few hours after infection.

One hour after infection a culture was isolated and a positive RIP obtained from the following organs: the liver, spleen, kidney and lungs. A positive RIP and negative bacteriological investigation occurred after 3 hrs in the spleen, mucosa of the ascending and descending colon, after 48 hrs – from the mucosa of the ascending colon and after 72 hrs – from the lymphatic glands. A positive culture and negative RIP were obtained from the mucosa of the descending colon and the bone marrow after 48 hrs (Table 3).

The results obtained show that with a higher concentration of bacterial bodies the spread of microorganisms throughout the body after oral infection can more easily be detected. In addition it was confirmed once more that spread of microorganisms evidently takes place by the hematogenous route within a few hours of infection.

Summarizing our experimental results from all 4 groups of rabbits, we come to the following conclusion: of the total number of analyses (360) 41.3% gave a positive result (both with bacteriological examination and RIP). Of this number, 61 analyses showed agreement between the results, which amounted to 67.7%; 25 analyses were found to show a positive RIP alone, and a negative bacteriological examination (27.8%) and 4 analyses resulted in a positive culture and a negative RIP (4.4%).

The experiments showed the promising nature of the RIP in the study of experimental problems of the pathogenesis of dysentery. In this work several features of the spread of dysentery bacteria in the bodies of infected rabbits were revealed as a result of the simultaneous performance of two methods of investigation, showing in particular the elimination of dysentery organisms from the blood stream to the mucosa of the intestine within a few hours of intravenous infection, the prolonged stay of the organism in the intestinal mucosa, the possibility of hematogenous dissemination after oral infection and so on.

SUMMARY

Data on experimental study of the possibility of using the reaction of phage titer increase (V. D. Timakov's and D. M. Gol'dfarb's method) for indication of dysentery microorganisms in the organism of infected rabbits is presented. This reaction is based on increase of the titer of the highly sensitive indicator phage in its interaction with the dysentery bacilli in the substrate under investigation. The principle of this reaction allows detection of the causative agents in very small concentrations within a relatively short period of time.

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ONCOLOGY

THE SIGNIFICANCE OF HIGHER NERVOUS ACTIVITY IN THE APPEARANCE AND DEVELOPMENT OF TUMORS OF THE BREAST IN MICE

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There are several reports in the literature of studies of the relationship between growth of tumors in various animals and aspects of higher nervous activity.

These studies may be divided into 2 groups: the first group consists of investigations of the appearance of tumors in animals after trauma to the nervous system [3, 5, 6, 10]; the second group is made up of investigations in which tumor growth was studied in animals with normal higher nervous activity [4, 8].

In earlier investigations on mice of the C_{57}-black strain, injected subcutaneously with a carcinogenic compound, we showed that in animals with mobile nervous processes, induced tumors appeared less often than in animals with inert nervous processes. On the basis of these findings we suggested that there is an essential relationship between the degree of mobility of its nervous processes and the resistance of an animal to carcinogenic agents.

The present investigation, a continuation of these earlier studies, was carried out on female mice belonging to the high-cancer strain C_3HA, a considerable proportion of which develop "spontaneous" tumors of the breast [7], i. e., tumors due to estrogenic stimulation and to the so-called milk factor with a definite hereditary susceptibility.

EXPERIMENTAL METHOD

In the experiment 112 females of the C_3HA strain were used, in 50 of which (47.7%), aged between 7 and 12 months, tumors of the breast had developed, while 62 mice were without tumors. Of these 112 mice, 96 (aged from 7 to 12 months) were investigated by the alimentary conditioned reflex method [1, 2, 9]. Before investigation all the animals received a diet in excess of normal requirements. During the investigation (2-4 months) they were put on a normal diet (25 calories per mouse per day with protein accounting for 18% of the calorific value), while at the end of the investigation they were formed into groups of 4-6 animals and again put on the ordinary diet.

The physiological investigation of the mice consisted of production of 2 conditioned reflexes (positive – to a bell, and inhibitory – to a light) and the bilateral modification of these reflexes. The mobility of the nervous processes was measured by the rate of modification of the reflexes in these mice, the criteria for the assessment of this property of the nervous system being: 1) the onset of modification, i. e., that state of conditioned reflex activity of the animals in which for the first time the reflex in response to a reinforced stimulus was greater in magnitude than the reflex to the stimulus when not reinforced by food, 2) the end of modification, characterized

by prolonged (not less than 5 successive experiments) dominance of the positive over the inhibitory reflex, and 3) total modification, in which the magnitude of the positive and inhibitory conditioned reflexes regained its initial value as it was before modification.

After 5 months all the experimental animals were subjected to regular inspection – observations were made of the time of appearance of tumors of the breast, the situation and number of tumor nodules. After death, all the animals were examined and the tumors and the lungs, containing metastases, examined microscopically. Of the 50 mice which developed tumors, multiple nodules were observed in 23 (46%) and metastases of the tumor in the lungs were found in 14 mice (28%).

The majority of the tumors of the breast were adenocarcinomas and small-cell intraacinous carcinomas, or tumors of mixed structure. Solid carcinomas, cystadenocarcinomas and adeno-acanthomas were observed in isolated cases.

Some of the mice (numbering 49) from the age of one month and before the beginning of the investigation (7-12 months) were kept in individual cages measuring 10 × 10 × 20 cm, and the remaining 63 mice in groups of 8-26 mice in large cages. These two groups differed sharply in the degree of mobility of their nervous processes: in the animals kept separately the mobility of the nervous processes was found to be on the average twice as high as that of the mice living in groups (12 experiments were required to modify the reflexes in the "individual" mice and 20 experiments for the "group" mice). As regards the proportion which formed tumors, these groups showed no difference from each other: in the first group tumors appeared in 40.5% of cases and in the second group in 40%.

EXPERIMENTAL RESULTS

The difference in the keeping of the animals, and in particular the difference in the average degree of mobility of the nervous processes, makes it essential to make a separate analysis of the results obtained from the 2 groups of mice mentioned.

TABLE 1

Statistical Treatment of the Results of Mobility of Nervous Processes in Healthy and Tumor-Bearing Mice

Modification of conditioned reflexes	Mice without tumors			Mice with tumors		
	no. of specimens	$M_1 \pm m_1$	coeff. of variability, %	no. of specimens	$M_2 \pm m_2$	coeff. of variability, %
	Mice kept separately					
Onset of modification	25	4.56±0.42	45	17	5.00±0.6	49
End of modification	24	11.04±1.00	44	17	13.00±1.8	66
Total modification	24	14.45±1.40	47	17	17.82±3.0	70
	Mice kept together					
Onset of modification	32	4.19±0.47	63	22	6.1±0.9	70
End of modification	31	17.39±1.7	55	21	23.7±4.31	84
Total modification	31	22.7±2.2	54	21	28.5±4.5	68

In Table 1 are shown the results, treated statistically, of experiments on mice kept separately. It is clear from these results that by all criteria the mobility of the nervous processes among unaffected animals is higher than in the group of animals with tumors. This difference is slight, however, not statistically significant, and may only be detected as a tendency towards higher mobility of nervous processes in unaffected mice. Here also are indicated the results of experiments on mice kept together. As can be seen, in the mice with tumors the mobility of the nervous processes is considerably less than in the group of animals without tumors. These differences are more marked than in the animals kept separately, nevertheless they are not statistically significant.

On comparing these results the following problems naturally arise. Why are the differences in the degree of mobility of nervous processes between unaffected and affected mice more pronounced in the animals kept together in groups? Why, in spite of the quite large variations in the degree of mobility of nervous processes between affected and unaffected animals kept in groups, are these differences not statistically significant?

In regard to the first question it must be stated that in mice kept separately the mobility of nervous processes was found to be considerably higher than in animals kept together; this could to some extent equalize the differences in this particular property of the nervous system between affected and unaffected animals. The answer to the second question may be found in the degree of variability of the results obtained. While in the group of unaffected animals the coefficient of variability is 66%, in the group of mice which developed tumors it is 84% (for cases of total modification of reflexes). It may easily be calculated that if the mobility of nervous processes in the group of affected animals varied to the same extent as in the unaffected group, the differences in the degree of mobility between these two groups of mice would be statistically significant.

We shall consider this last question in somewhat greater detail.

In Table 1 results are shown which also illustrate the degree of variability of nervous processes in affected and unaffected animals. It can be seen from these figures that in every case the variability of this particular property is considerably higher in mice with tumors than in animals without. This fact can be accounted for in two ways. In the first place there is greater homogeneity in the properties of the nervous system, especially in the strength of the nervous processes, in mice with a higher mobility of the nervous system, i. e., in the mice unaffected by tumors, while among animals with tumors and with a more inert nervous system the higher nervous activity is of a type which shows extremes of strength. In the second place it has to be pointed out that the mice were studied at a period when some of them were beginning to develop malignant neoplasms. It might be suggested that the presence of tumors in certain animals was a factor which increased the variability of our findings, especially in view of similar results in the work of N. M. Turkevich. However, if all the animals with tumors are divided into 2 groups (those examined before tumors were discovered and those with tumors), and comparing their degree of variability of mobility of nervous processes, we observed significant differences, although in the opposite direction: in investigation of mice with tumors the coefficient of variability was 81%, and in investigation of mice before tumors were found — 91%. No difference was found in the degree of mobility of nervous processes between these groups of animals: in the first group the end of modification of the reflexes occurred on the average after 20.2 ± 3.9 experiments, and in the second group — after 18.2 ± 3.6 experiments. Thus the presence or absence of tumors at the time of the investigation of higher nervous activity is not the main cause of the lowered mobility and the increased variability of the results in the affected mice.

TABLE 2

Mobility of Nervous Processes in Various Groups of Mice Kept Separately and in Groups

Group of mice	No. of mice	Average duration of life in days	Mobility of nervous processes in mice kept	
			separately	together
With solitary tumor nodules without metastases	13	48	4.15-12.92-16.38	4.7-18.4-28.5
With multiple tumor nodules without metastases	12	44		
With solitary tumor nodules and metastases in the lungs	6	103	5.75-15.75-22.5	8.16-23.5-33.16
With multiple tumor nodules and metastases in the lungs	7	94		

From the results shown in Table 2 it can be seen that metastases from the breast tumors in the lungs were observed in those mice which survived long enough. Analysis of the degree of mobility of nervous processes in animals dying with and without metastases showed that the mobility of nervous processes was lower in the first than in the second, i. e., among mice with metastases there was a predominance of animals with more inert processes.

TABLE 3

Time of Appearance of Tumors and Length of Life of Affected Mice

Characteristics of the higher nervous activity of the animals	No. of specimens	Extreme variants	$M_1 + m_1$
Times of appearance of the primary tumor (in days)			
With mobile nervous processes	25	245-600	386.6 ± 15.0
With inert nervous processes	13	239-540	403 ± 30.7
Duration of life of animals with tumors (in days)			
With mobile nervous processes	25	0-123	47.0 ± 7.8
With inert nervous processes	13	0-164	57.0 ± 13.5

The facts which we obtained may be analyzed from the point of view of the time of onset of the primary tumors and the duration of life of the affected animals in relation to the degree of mobility of nervous processes. For this purpose all the affected mice were divided into 2 groups differing significantly in the degree of mobility of their nervous processes. The relevant data is shown in Table 3, from which it can be seen that tumors appeared somewhat sooner in mice with mobile nervous processes, and that these animals died after a shorter time than mice with an inert nervous system.

From a summing up of the material presented and a comparison with results obtained from earlier experiments with carcinogenic compounds of mice of the C_{57} strain the following conclusions can be drawn.

There is undoubtedly a relationship, which can be revealed by keeping and studying animals under certain conditions, between the degree of mobility of nervous processes as determined by the modification of two conditioned reflexes, and the resistance of the animals to factors causing malignant neoplasms. The same relationship also exists between metastasis formation and the degree of mobility of nervous processes, i. e., among mice with metastases from a tumor of the breast there is a predominance of mice with more inert nervous processes.

In spite of the fact that a smaller percentage of mice with higher mobility of nervous processes develop tumors, the tumors appear sooner, and the affected animals die rather more quickly than mice with a less mobile nervous system.

SUMMARY

The appearance and development of tumors of mammary glands in female mice of the highly-cancerous line C_3HA was studied. The peculiarities of the higher nervous activity were investigated with the aid of the motor food method (E. A. Ganike – 1935, 1939 and 1941 and V. K. Fedorov – 1951). The following was established.

1. There is a direct relationship between the degree of mobility of the nervous processes and the resistance of the organism to the "milk factor." This alone was detected only in definite conditions of keeping and investigation of animals.

2. The same relationship exists between the process of metastasis and the degree of mobility of the nervous processes, i. e., mice with inert nervous processes prevailed among the animals with metastases of the tumors.

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THE LEUKEMIA-PRODUCING ACTIVITY OF CELL-FREE FILTRATES OF HUMAN LEUKEMIC TISSUE

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The ability to pass through a semipermeable membrane is a characteristic property of the so-called filtrable viruses. By the use of special filters it is possible to isolate from a tissue extract a virus component, leaving undamaged cells and bacterial microorganisms on the filter [2]. If injection of the filtrate into an animal causes the appearance of a disease, this implies that the filtrate contains a virus.

The possibility of transmission of a tumor from an affected animal to a healthy one by means of a filtrate is the main factor in support of the virus etiology of these tumors. Tumors of this sort include carcinoma of the breast and leukemia in mice, papilloma and fibroma in rabbits, fibroma in deer, sarcoma and leukemia in fowl and so on.

There are no indications in the literature of the possibility of transmission of leukemia in man by means of cell-free filtrates. We have produced leukemia in mice by injecting them with centrifuged extracts of human leukemic tissue in which the presence of whole cells was practically impossible. However, the objection that isolated undamaged cells may still be left in the extract persists.

For this reason it was essential to attempt to produce leukemia in mice by means of cell-free filtrates of human leukemic tissue. In the present paper we give the results of experiments undertaken in this direction.

EXPERIMENTAL METHOD

We prepared cell-free filtrates from tissue from lymphatic glands, blood, brain and tumor-like leukemia infiltrations from 4 human patients suffering or dying from acute leukemias (hemocytoblastoses). In control experiments we used extracts of donated blood and of brain tissue from a patient dying from vascular disease. We had studied previously the biological activity of extracts of "normal" lymphatic glands: the extracts possessed no leukemia-producing activity [1]. In the same report the findings are given of the leukemia-producing activity of extracts of human sarcoma (as a control of tumor-like leukemia infiltration).

* In Russian.

The filtrates were prepared as follows: the tissue was ground up in a blender in the cold with physiological saline in a proportion of 1:5; the suspension was strained through gauze and then centrifuged at 2500-3000 rpm for 10-15 min. The supernatant fluid was filtered through a Seitz apparatus at a negative pressure (oil pump) with a two-layer asbestos filter. The filtrates were tested for sterility.

In one case, after preparation of filtrates from brain tissue and a tumor-like leukemic infiltration from a patient dying from acute hemocytoblastosis (chloroma) we changed the method of obtaining the filtrate; the suspension contained 10% of 96% alcohol and before centrifuging it was allowed to stand at 4° for 20 hrs. It was then filtered through a No. 3 Rublevskii filter (diameter of pores 700 mμ).

The filtrates were injected into mice of the low leukemic strains C_{57} and C_3HA and impure strains, directly into the tissue of the spleen (adult mice) or subcutaneously (newborn). Altogether 309 mice were used in the experiments. Animals injected with filtrates of leukemic brain tissue and tumor-like infiltrates were given 5 mg of cortisone one month later (in 4 doses at intervals of 2-3 days). Blood from these mice was inoculated in nutrient media in order to detect paratyphoid infection which may give severe leukemoid reactions. No paratyphoid bacteria were discovered.

The diagnosis of true leukemia was made on the basis of the careful histological and cytological study of organs of the experimental animals. Where necessary the true malignant nature of the lesion was confirmed by transplantation into other mice of the same strain.

EXPERIMENTAL RESULTS

Essential details of the experimental results are shown in the summarized table.

Summarized Table of the Experiments

Extract	Age of mice	Method of injection of extract	No. of mice used in the experiment	Time of first appearance of leukemia	No. of mice surviving to time of 1st appearance of leukemia	Average latent period of development of leukemia in months	No. of leukemic mice	No. of leukemoid reactions
Filtrate of leukemic brain	1.5 month	Intraperitoneal	21	3	19	4.7	5	3
	7 days	The same	8	5	4	5	1	0
The same, in 10% aqueous alcohol	1.5 month	Into spleen	41	1.5	22	2.3	5	6
	1-6 days	Subcutaneous	28	5	3	5	1	4
Filtrate of normal brain in 10% aqueous solution	1 month	Into spleen	40	2.5	25	2.5	1	9
	3-14 days	Subcutaneous	21	–	4	–	0	1
Filtrate of leukemic tumor	1.5 month	Into spleen	19	2.5	8	3	2	3
Filtrate of leukemic blood	5-14 days	Subcutaneous	23	1	14	1	1	2
	5 days	"	33	6	4	6	1	5
Whole donated blood	1 month	Into spleen	26	–	20	–	0	4
Filtrate of leukemic lymphatic glands	1 month	Into spleen	9	1	8	1.5	2	1

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ANALYSIS OF THE MECHANISM OF DEGRADATIONAL MITOGENETIC RADIATION

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Degradational mitogenetic radiation arises in living objects during moderate cooling (2-5°C), light anesthesia, centrifugation (2500-3000 rpm), the application of a weak (0.02-0.05 ma at 4-6 v) constant or alternating current, or spontaneously, as, for example, in nerve and muscle systems [1, 2, 3].

An analysis of this phenomenon led A. G. Gurvich to the conclusion that in the substance of living objects there are molecular constellations at a high energy level for the maintenance of which statistically uninterrupted influx of energy is required, and that consequently these molecular constellations are not in equilibrium.

According to Gurvich [4], these unbalanced molecular constellations consist of general levels of energy along which absorbed energy migrates and possibly is summated by the constellations. During disturbance of the constellations this energy is partially emitted in the form of degradational radiation, with a spectrum which is determined by the unbalanced state of the molecular constellations. According to A. G. Gurvich's physiological theory of protoplasm [3], the unbalanced molecular constellations constitute the fundamental reactive apparatus of living systems.

We thus see that the importance of studying degradational mitogenetic radiation lies in the fact that first by its appearance or absence during the application of degrading factors to a living object it is possible to judge the intrinsic energy level of that object; in the second place the spectrum of this radiation may give some idea of the state of the general reactive apparatus of living systems – on the state of the unbalanced molecular constellations and on the mitogenetic condition of the nerve and muscle systems.

The above indicates the considerable general biological importance of the study of degradational mitogenetic radiation. However, in the mechanism of its production there is much that still remains unexplained.

The investigation to be described was devoted to the study of the duration of degradational radiation and of the ability of the unbalanced molecular constellations to accumulate energy and to summate small quanta of it. As the subject of the investigation we used the degradational radiation of the liver of the mouse and of the layer of an onion, caused by cooling, and the method of demonstrating it was by the use of biological detectors.

1. Mouse liver. Duration of degradational radiation in relation to the temperature of cooling.

The degradational radiation of the liver during cooling was studied by A. and L. Gurvich [1, 2] and by Iu. N. Ponomareva on the transplanted extirpated organ; we studied the liver in situ.

EXPERIMENTAL METHOD

The mouse, with its liver exposed (the operation was performed without anesthesia), was covered by a screen with an opening situated over the liver.

The liver was irrigated with physiological saline at 38-41°C, and at the end of 10 min its radiation was tested; next the warm solution was replaced by cold (also physiological saline) at 6-9°C and 0-3°C, and the radition from the liver again tested. This test was carried out quickly, soon after the start of the irrigation with cold water, and lasted for 10 sec (exposure). The next exposure began 5 sec after the end of the previous one; it also lasted for 10 sec. If, for example, on the fourth exposure mitogenetic effect was found to be absent, then the next test (on the same animal) began 55 sec after the start of irrigation with cold physiological saline, and the exposure was increased to 15 sec, and so on.

Duration of degradation in the liver of the mouse. During cooling of the mouse liver to 6-9°C its degradation radiation continued for about 6 min, and in order to obtain any mitogenetic effect at the end of this period an exposure of 40 sec was required. After the lapse of 6 min no mitogenetic effect could be obtained with exposures of 45, 50, or even 55 sec. During cooling to 3.5-0°C the degradation radiation of the mouse liver lasted for about 7 min, while in order to obtain mitogenetic effect at the end of this period an exposure of 35 sec was necessary. In this series of experiments the limits of the duration of degradational radiation were not reached (Tables 1a and 1b).

TABLE 1a

Induction from the Liver of a Live Mouse During Irrigation of the Liver with Warm and Cold Physiological Saline (series I)

Exposure in seconds	Effect in % *	Temperature of solution in °C	Number of experiments
10	−1	39	3
40	−3	41-42	3
10 with an interval of 0 to 115**	37	6-9	10
15-25 with an interval of 30 to 250	42	5-8	5
30-40 with an interval of 255 to 380	36	7-9	5
45-55 with an interval of 380 to 435	4	8-9	5

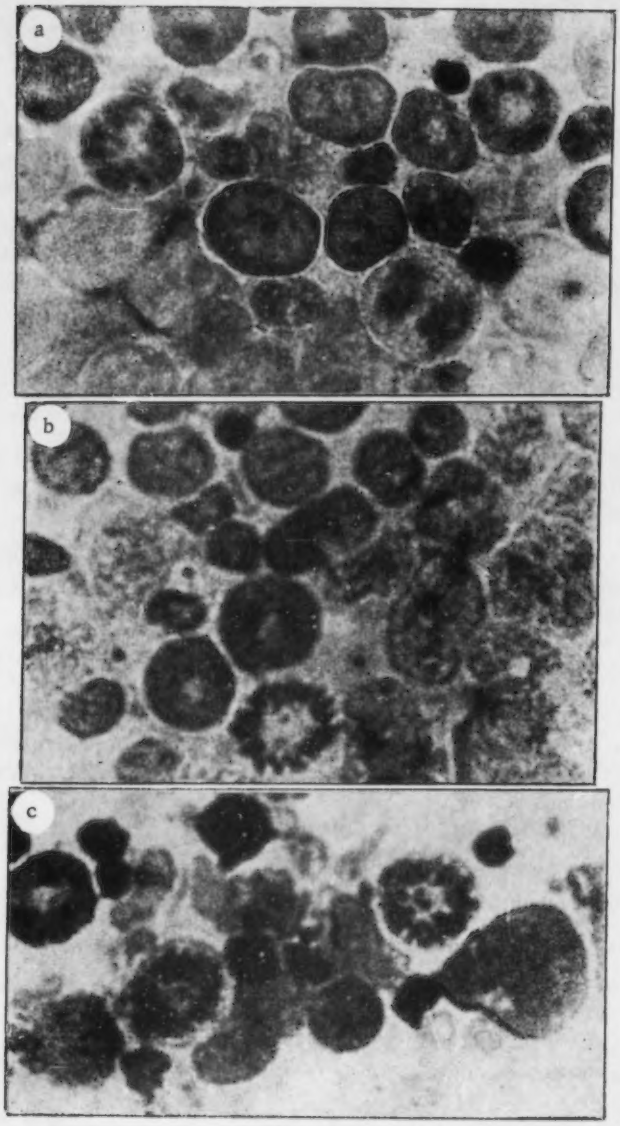
* In this and subsequent tables: effect in % is calculated by the formula: $\frac{E-C}{C} \cdot 100$, where E is experiment, C is control.

** In this and subsequent tables the expression "exposure" 10 sec with an interval of 0 to 115 sec means that the given exposure may be taken anywhere during the time interval starting from the moment of irrigation with cold physiological saline to 115 sec from this zero.

TABLE 1b

Induction from the Liver of a Live Mouse During Irrigation of the Liver with Warm and Cold Physiological Saline (series II)

Exposure in seconds	Effect in %	Temperature of solution in °C	Number of experiments
5-10	2	40-41	6
15-20	−4	40-42	3
25-35	2	39-42	3
5-10 with an interval of 0 to 115	37	0-4	14
12-20 with an interval of 115 to 250	40	0	8
25-35 with an interval of 250 to 440	42	0	6



Cellular composition of the bone marrow and spleen of a mouse after injection with a cell-free filtrate of human leukemic brain tissue. a, b) Bone marrow films; c) spleen film. Pappenheim's stain.

It will be seen from the table that cell-free filtrates of human leukemic tissues are undoubtedly able to produce leukemia in mice of a low leukemic strain. Leukemia developed in 25% of the animals surviving for the period of appearance of primary leukemia, their average latent period of development being 3.7 months. In a rather smaller percentage (11) leukemia blood.

The first cases of leukemia were observed from 1-1.5 months after the beginning of the experiment (they were confirmed by positive transplantation results). In their morphological structure the leukemias were of the myeloid type (see figure a,b,c).

In the control experiments only one case of true leukemia (3.4%) was found after the use of a filtrate (in 10% aqueous alcohol) of "normal" brain tissue. A leukemoid reaction was observed equally often in the experimental and control series. It must be mentioned that attempts to transplant organs of mice with severe myeloid reactions did not lead to the development of true leukemias.

On injection of newborn mice with filtrates of leukemic blood, we observed in those mice which died 2 weeks after the beginning of the experiment an increased content of immature cells in the bone marrow, with a normal composition of the spleen and liver. A similar phenomenon has been observed by Czech workers in young rats injected with leukemic cells. This is the so-called transitory "phase of blasts" [4].

The high leukemia-producing activity of filtrates of human brain tissue must be emphasized. This fact has also been pointed out by other workers [3, 5], but in relation to extracts of the brain of leukemic mice.

At present it is still difficult to account for such a high specific activity of extracts of leukemic brain; we think that it is concerned with the high lipoid content of this tissue, for lipoids play an essential part in the realization of the biological action of the "leukemia factor."

The results of the experiments just described, it seems to us, make it possible to include leukemia in man among the malignant neoplasms which are transmissible by means of cell-free filtrates. This illustrates the virus-like properties of the etiological factor of leukemia in man.

SUMMARY

Cell-free filtrates of leukemic human tissues were administered to mice of low leukemic lines. True leukoses developed on the average in 18% of mice, which survived the period of appearance of the first case of leukosis (with the average latent period of development of leukosis equal to 3.7 months). Extracts of "normal" human tissues do not possess such activity.

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EXPERIMENTAL BIOLOGY

A STUDY OF THE PROLIFERATIVE PROPERTIES OF CORNEAL TISSUES DURING WOUND HEALING

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The cornea of the eye has long been considered by scientists to be a valuable subject for the study of many biological problems [12, 13, 21, 23].

The theory that regenerating epithelium is unilaminar before it becomes stratified [17, 19, 24] has been critically analyzed by N. A. Shevchenko [16] and can now be considered baseless. However, many things regarding the structure of regenerating epithelium and especially of its growth edge, are not yet sufficient clear.

Epithelial proliferation is regarded by some authors as an active process [12, 16, 24, 27] and by others as a passive process [1]. Some authors do not believe that the whole epithelial sheet is displaced; these authors believe that regeneration is caused by the movement of the basal cells alone to the top of the sheet [22]. There is a theory that epithelial regeneration is caused by the disintegration of the sheet into cellular elements which have a reproductive or defense-support function [2, 3, 18].

According to some authors, the actual substance of the cornea is regenerated both from the local connective tissue cells and from the cells emigrating from the eye tissues surrounding the cornea [4, 6, 8]. Other authors [19, 20] give more significance to either the connective tissue cells of the limbus, which migrate to the focus of regeneration, or the local corneal cells [9, 10, 11]. There was another theory expressed by certain authors [14, 25, 27] some time ago that the connective tissue cells of the cornea formed from the epithelium.

Although many authors [4, 7, 9, 16] admit that epithelium is more active than connective tissue, it has also been shown that the tissues must proliferate simultaneously [17] and that there must be a definite correlation between the regeneration of the epithelial tissue and that of the connective tissue [8]. The theory that differentiation of the corneal epithelium is associated with the development of Bowman's membrane [5] is not sound, since a structurally expressed Bowman's membrane does not exist in some animals.

EXPERIMENTAL METHOD

The experiments were done on 62 rabbits. First, the cornea was anesthetized with a 2% solution of cocaine hydrochloride and irrigated with a weak solution of boric acid; then a wound was inflicted with a trephine as much in the center of the cornea as possible. The epithelium and the connective tissue were injured; the corneal defects were made in the form of holes 2 mm in diameter. The cornea was examined 1, 2, 4, 6, 12 and 18 hours as well as 1, 6, 8, 14 and 30 days after the operation. The material was fixed in Zenker's fluid. The paraffin and celloidin sections were mostly stained with Heidenhain's iron hematoxylin with an after-stain of Mallory's mixture or Bemer's hematoxylin with picroindigocarmine.

EXPERIMENTAL RESULTS

The first reaction of the tissues to the wound consisted of disturbance in the metabolic processes and the development of a reactive inflammation, more or less expressed. In the epithelium this reaction was characterized

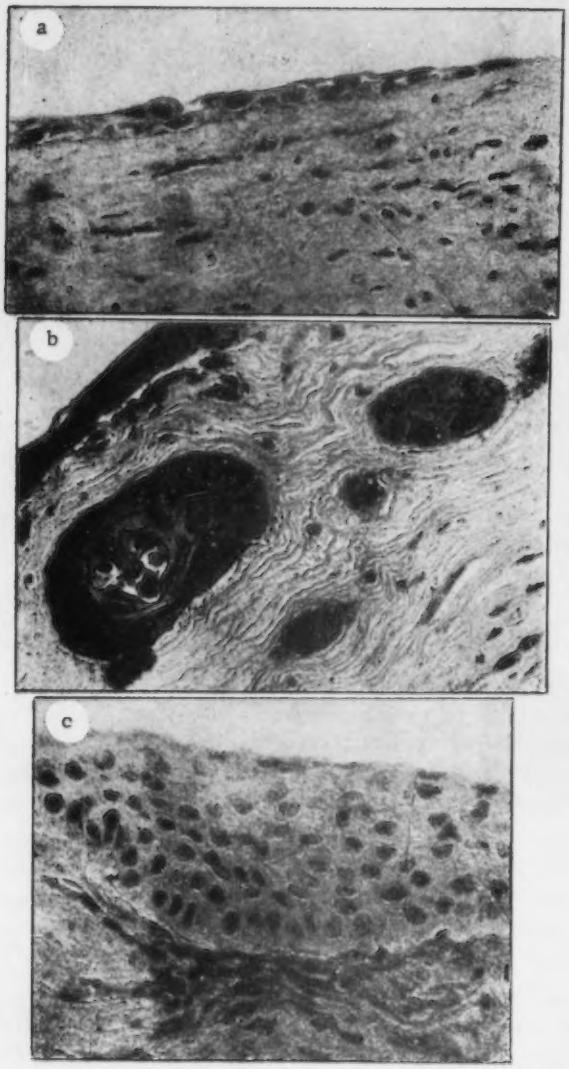

Fig. 1. The process of proliferation of the epithelium of the cornea in a rabbit. a) Single-layer wedge of regenerating epithelium on destroyed connective tissue 4 hours after operation. Fixative: Zenker's fluid. Stain: Carazzi's hematoxylin and eosin. Magnification: apochromatic obj: 2 mm, comp., ocular 5 x. b) Ingrowth of epithelium into connective tissue in the form of a band 4 days after operation. Fixative: Zenker's fluid. Stain: iron hematoxylin. Magnification: apochromatic obj: 2.2 mm, comp., ocular 5 x. c) Accumulation of fibroblasts under newly formed epithelium. 6 days after operation. Fixative: Zenker's fluid. Stain: Bemer's hematoxylin and picro-indigocarmine. Magnification: apochromatic obj: 2.2 mm, comp., ocular 5 x.

morphologically by a disturbance in the stratification. Some of the less differentiated cellular elements became activated and lost any sign of functional differentiation. Other elements, which had already started the differentiation process and were incapable of activation, quickly degenerated. The theory that this process consists of two phases – first, the formation of a unilaminar epithelial regenerate and then its transformation into stratified epithelium [17, 19, 24] – was not confirmed in the experiments conducted. But the actual wedge of epithelial growth often consisted of only a few layers, its edge sometimes even being unilaminar (see figure).

During the proliferation of the epithelial sheet the tissue connections were weakened and the intercellular relationships changed. The edge of the epithelial sheet became wedge-shaped (see figure). At the beginning of proliferation the shape of the edge depended both on the character of the wound and on the type of reorganization, a phenomenon connected with the degree of activation.

Later in the proliferation process the degree of stratification depended on the intensity of the epithelial growth. The faster the growth of the epithelium, the thinner its edge. At first epithelization was due primarily to activated cells from the surrounding epithelium causing the edge of the latter to become much thinner. Changes in the shape of the cellular elements also caused the epithelium to become thinner in the marginal zone.

Most of the cells divided by mitosis; division occurred first in the unchanged epithelium, with mitoses being found in the activated zone of the epithelium only 10-12 hrs later some distance from the edge of the regenerate. The migration of cambiogenetic* cells into the marginal zone described by some authors [2, 3, 17, 18] does not occur in actual fact.

Although the connective tissue of the cornea was less reactive (in the sense of its proliferative properties), considerable changes were observed in it from the moment the wound was inflicted. Edema and leukocyte infiltration developed, disturbing the laminar distribution of the collagenous fibers.

The activated epithelium grew over and smoothed out this uneven connective tissue.

Under conditions of wound regeneration it is comparatively difficult to observe the relationship between the differentiation of newly formed epithelium and the condition of the underlying tissue. The epithelial regenerate began differentiation very early. The new epithelium began to differentiate at the 24-hr stage, before the wound was completely epithelized and before connective tissue proliferation had started; a basal layer of tall cells and a second layer of polymorphic and flat cells could be discerned in it. These data contradict the theory [5] that epithelial differentiation begins after the appearance of Bowman's membrane. We found that the epithelium could lie either on the nonproliferating connective tissue or on the newly formed connective tissue and have in either case a definite laminar polarity. A definite relationship with the underlying tissue is evidently established during epithelial differentiation, the disturbance of which can cause the epithelium to grow into the underlying tissue (see figure, b).

The main substance of the cornea was regenerated from the connective tissue cells of the cornea proper. However, the connective tissue cells of the limbus could also participate in the regeneration process, especially when the corneal defect was near the limbus. Therefore, it would seem that in the cornea there were both differentiated corneal connective tissue cells (fibrocytes) and less differentiated cells [5, 17], which reacted to the wound in different ways. The fibrocytes of the injured area disintegrated. Activated fibroblasts migrated from the uninjured area under the connective tissue surface into the regenerating connective tissue. This was attended by the constant dehydration of the regenerating tissue and by a decrease in the number of leukocytes. The number of fibroblasts increased and accumulated under the regenerated epithelium (see figure, c). The fibroblasts divided both mitotically and amitotically; division was first observed 48 hrs after the operation.

The old collagenous fibers disintegrated in the sections where the fibroblasts had accumulated, and new intercellular fibrous structures formed. The disintegration of the old collagenous fibers, the special leukocytes [17] participating, began with the inflammatory process and subsequently proceeded in the presence of lymphocytes and histiocytes.

The zone of the disintegrating corneal connective tissue was gradually filled with newly formed tissue. For a long time this regenerate consisted of intercellular matter and cells of the fibroblast type. The laminar structure was not expressed; the newly formed fibrous structures, in the form of fibrils and bundles, were unorientated and remains of the old fibers were found among them.

* As in original.

Therefore, the regenerated portion of the cornea had not yet acquired the structure typical of an uninjured cornea at the latest stages of regeneration which we examined.

SUMMARY

The process of healing of the injured cornea was investigated in rabbits. This process was followed up for the period ranging from 1 hr to 30 days after infliction of the injury. Biphasic course of regeneration of the epithelium and migration of cells from the epithelial layer to the border zone was not revealed. It was demonstrated that the structure of the epithelial wedge depended on the conditions of the experiment. Connective tissue possesses a lower reactivity than epithelium and it regenerates with its own cells. When the wound is inflicted near the limbus the elements of the latter may take part in its regeneration.

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ANALYSIS OF THE MECHANISM OF DEGRADATIONAL MITOGENETIC RADIATION

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Degradational mitogenetic radiation arises in living objects during moderate cooling (2-5°C), light anesthesia, centrifugation (2500-3000 rpm), the application of a weak (0.02-0.05 ma at 4-6 v) constant or alternating current, or spontaneously, as, for example, in nerve and muscle systems [1, 2, 3].

An analysis of this phenomenon led A. G. Gurvich to the conclusion that in the substance of living objects there are molecular constellations at a high energy level for the maintenance of which statistically uninterrupted influx of energy is required, and that consequently these molecular constellations are not in equilibrium.

According to Gurvich [4], these unbalanced molecular constellations consist of general levels of energy along which absorbed energy migrates and possibly is summated by the constellations. During disturbance of the constellations this energy is partially emitted in the form of degradational radiation, with a spectrum which is determined by the unbalanced state of the molecular constellations. According to A. G. Gurvich's physiological theory of protoplasm [3], the unbalanced molecular constellations constitute the fundamental reactive apparatus of living systems.

We thus see that the importance of studying degradational mitogenetic radiation lies in the fact that first by its appearance or absence during the application of degrading factors to a living object it is possible to judge the intrinsic energy level of that object; in the second place the spectrum of this radiation may give some idea of the state of the general reactive apparatus of living systems – on the state of the unbalanced molecular constellations and on the mitogenetic condition of the nerve and muscle systems.

The above indicates the considerable general biological importance of the study of degradational mitogenetic radiation. However, in the mechanism of its production there is much that still remains unexplained.

The investigation to be described was devoted to the study of the duration of degradational radiation and of the ability of the unbalanced molecular constellations to accumulate energy and to summate small quanta of it. As the subject of the investigation we used the degradational radiation of the liver of the mouse and of the layer of an onion, caused by cooling, and the method of demonstrating it was by the use of biological detectors.

1. Mouse liver. Duration of degradational radiation in relation to the temperature of cooling.

The degradational radiation of the liver during cooling was studied by A. and L. Gurvich [1, 2] and by Iu. N. Ponomareva on the transplanted extirpated organ; we studied the liver in situ.

EXPERIMENTAL METHOD

The mouse, with its liver exposed (the operation was performed without anesthesia), was covered by a screen with an opening situated over the liver.

The liver was irrigated with physiological saline at 38-41°C, and at the end of 10 min its radiation was tested; next the warm solution was replaced by cold (also physiological saline) at 6-9°C and 0-3°C, and the radition from the liver again tested. This test was carried out quickly, soon after the start of the irrigation with cold water, and lasted for 10 sec (exposure). The next exposure began 5 sec after the end of the previous one; it also lasted for 10 sec. If, for example, on the fourth exposure mitogenetic effect was found to be absent, then the next test (on the same animal) began 55 sec after the start of irrigation with cold physiological saline, and the exposure was increased to 15 sec, and so on.

Duration of degradation in the liver of the mouse. During cooling of the mouse liver to 6-9°C its degradation radiation continued for about 6 min, and in order to obtain any mitogenetic effect at the end of this period an exposure of 40 sec was required. After the lapse of 6 min no mitogenetic effect could be obtained with exposures of 45, 50, or even 55 sec. During cooling to 3.5-0°C the degradation radiation of the mouse liver lasted for about 7 min, while in order to obtain mitogenetic effect at the end of this period an exposure of 35 sec was necessary. In this series of experiments the limits of the duration of degradational radiation were not reached (Tables 1a and 1b).

TABLE 1a

Induction from the Liver of a Live Mouse During Irrigation of the Liver with Warm and Cold Physiological Saline (series I)

Exposure in seconds	Effect in % *	Temperature of solution in °C	Number of experiments
10	−1	39	3
40	−3	41-42	3
10 with an interval of 0 to 115**	37	6-9	10
15-25 with an interval of 30 to 250	42	5-8	5
30-40 with an interval of 255 to 380	36	7-9	5
45-55 with an interval of 380 to 435	4	8-9	5

* In this and subsequent tables: effect in % is calculated by the formula: $\frac{E-C}{C} \cdot 100$, where E is experiment, C is control.

** In this and subsequent tables the expression "exposure" 10 sec with an interval of 0 to 115 sec means that the given exposure may be taken anywhere during the time interval starting from the moment of irrigation with cold physiological saline to 115 sec from this zero.

TABLE 1b

Induction from the Liver of a Live Mouse During Irrigation of the Liver with Warm and Cold Physiological Saline (series II)

Exposure in seconds	Effect in %	Temperature of solution in °C	Number of experiments
5-10	2	40-41	6
15-20	−4	40-42	3
25-35	2	39-42	3
5-10 with an interval of 0 to 115	37	0-4	14
12-20 with an interval of 115 to 250	40	0	8
25-35 with an interval of 250 to 440	42	0	6

2. Onion layer. Duration of degradational radiation in relation to the initial temperature of the object.

In order to obtain degradational radiation the onion layer was pressed between two discs of ice (ice discs 2-3 mm thick transmit mitogenetic radiation). Control experiments in which blocks of yeast were placed on ice discs (without onion layers) and given exposures of 10, 15 and 20 sec showed that the ice discs did not affect the biodetector in the conditions of the experiment (mean effect 5%).

TABLE 2

Induction from the Layer of an Onion (Initial Temperature 9-12°C) During Cooling

Exposure in seconds	Effect in %	Number of experiments
10 with an interval of 0 to 40	42	8
15-20 with an interval of 40 to 65	3	7

Initial temperature of the onion layer 9-12°C. At experimental temperatures of 9-12°C the onion layer showed no obvious activity, and during irrigation with water at this temperature it did not emit radiation (exposures of 10, 15 and 25 sec). The degradation radiation of the onion layer causes well-marked mitogenetic effects during an exposure of 1) sec (mean effect 40%) and it continues for about 40 sec. At the end of this period no mitogenetic effect can be obtained even with twice the exposure, i. e., 20 sec (Table 2).

A temperature of 9-12°C is close to the temperature of 6-9°C at which degradational radiation was produced in the liver, and so we assumed that under these conditions the unbalanced constellations of the onion layer had undergone partial degradation, which would perhaps explain the short duration of its degradational radiation. In subsequent experiments before cooling the onion layer was irrigated with water at a temperature of 25-27°C for a period of 10 min.

Initial temperature of the onion layer 25-27°C. Control experiments to test the enzymatic mitogenetic radiation of the onion layer during irrigation with water at a temperature of 25°C showed the absence of mitogenetic effect during exposures of 5 to 25 sec [mean (of 6 experiments) effects 4%] and that it appeared only during exposures of 30-50 sec [mean (of 6 experiments) effects 42%]. Cooling the onion layer after 7-30 min irrigation with water at a temperature of 25-27°C causes degradational radiation, detectable during an exposure of 10 sec and lasting about 100 sec. After the lapse of 100 sec, radiation is observed only with an exposure of 40 sec (Table 3).

TABLE 3

Induction from the Layer of an Onion (Initial Temperatures 25-27°C) During Cooling

Exposure in seconds	Effect in %	Number of experiments
10 with an interval of 30 to 105	46	12
10-30 with an interval of 105 to 135	1	6
40 with an interval of 105 to 145	37	1

TABLE 4

Exposure in seconds	Effects in %	Number of experiments
10 with an interval of 105 to 155	29	12
25 with an interval of 155 to 180	43	3
40 with an interval of 180 to 220	35	1

Initial temperature of the onion layer 35-37°C. The results of these experiments are shown in Table 4.

Since the duration of the degradational radiation is increased from 40 to 220 sec by a change in the initial temperature of the onion layer from 9-12°C to 35-37°C, it follows that in relation to the influx of energy the unbalanced constellations accumulate different quantities – they have a different "capacity." Of course there must be a limiting "capacity" of these constellations, and when this is reached the surplus energy which is absorbed

may possibly cause disturbance of the constellations. I remember that A. and L. Gurvich were unable to obtain degradational radiation from the liver after irradiating the organ for 20 min with mitogenetic rays. It is possible that further increase of the initial temperature might also lead to disturbance of the unbalanced constellations. For this reason we warmed the onion layer to 43°C with water and tested it for degradational radiation. This temperature might, however, prove lethal for the object. Therefore, in the experiments in which the initial temperature of the onion layer was 43°C, we always set up a special control in which the onion layer was transferred after cooling to water at a temperature of 35-37°C and, after the lapse of 10 min, it underwent a second cooling. Degradational radiation would indicate that the onion layer was undamaged (Tables 5 and 6).

TABLE 5

Induction from the Layer of an Onion (Initial Temperature 43°C) During Cooling

Exposure in seconds	Effect in %	Number of experiments
5-10 with an interval of 30 to 40	3	4
5-10 with an interval of 95 to 105	−2	3
5-10 with an interval of 155 to 165	2	3

TABLE 6

Induction from the Layer of an Onion Cooled After Heating to 43°C, Cooling and Reheating to 35-37°C

Exposure in seconds	Effect in %	Number of experiments
10 with an interval of 120 to 130	35	5

Tables 5 and 6 show that cooling an onion layer previously heated to 43°C cannot cause degradational radiation, and that the changes brought about by heating in this way are easily reversible, since after transfer of the cooled onion layer into water at a temperature of 35-37°C, the appearance of degradational radiation could be caused by cooling it a second time.

Degradational radiation from an onion layer having previously been cooled once. The onion layer was irrigated with water at a temperature of 27°C for 10 min, cooled for 2.5-3 min, again irrigated with water at the same temperature for 10 min and again cooled (Table 7).

TABLE 7

Induction from the Layer of an Onion Heated to 27° for 10 Minutes, Cooled for 3 Minutes, Reheated to 27°C for 10 Minutes and Again Cooled

Test	Exposure in seconds	Effect in %	Number of experiments
During reheating	10	−2	3
During cooling for the second time	10	50	3

The result is perfectly clear: absence of radiation during reheating, with an exposure of 10 min and a well-marked mitogenetic effect during the subsequent recooling with the same exposure for 10 sec.

Analysis of the production of photons of degradational radiation. The experiments described and results previously obtained [5] showing that a cancer suppressor does not suppress degradational radiation permit an experimental solution of the problem whether degradational radiation arises only in consequence of large quanta of energy, formed by the recombination of free radicals or atoms and absorbed by the unbalanced molecular constellations, or whether it may arise as a consequence of summation of small quanta of energy by the constellations.

In order to solve this problem it was necessary to prevent recombination of free radicals or atoms by injecting into the onion layer a hydrolyzed cancer suppressor and to get rid of the large quanta of energy already absorbed by the molecular constellations by means of degradation. For this purpose the onion layer was heated

in water at a temperature of 25-26°C for 10 min, its enzymatic radiation tested, and then immersed in a solution of the hydrolyzed suppressor at a temperature of 25-26°C for 10 min, and its enzymatic radiation again tested. Next the onion layer was cooled and during cooling its degradational radiation was tested. Cooling lasted for 2.5-3 min in order to be sure that the degradational radiation had completely ceased. After cooling, the same onion layer was again immersed in the suppressor solution at a temperature of 25-26°C for 10 min and its enzymatic radiation was tested; it was then recooled (Table 8).

TABLE 8

Induction	Exposure in seconds	Effect in %	Number of experiments
From the onion layer after heating to 25-26°C for 10 min	50	31	2
From the same onion layer after staying for 10 min in a solution of a hydrolyzed cancer suppressor at a temperature of 25-26°C	50	−2	2
From the same onion layer during cooling	10	23	2
From the same onion layer after reimmersion for 10 min in the suppressor solution at a temperature of 25-26°C and subsequent cooling for 3 min	50	3	2
From the same onion layer during the second cooling	10	35	2

In order to make sure that this five-stage operation in itself did not affect the onion layer, we made a control series of experiments. The results of these experiments showed that immersion of the onion layer five times in water at a temperature of 27°C had no effect on the enzymatic radiation from the layer (mean effect 29%).

Table 8 indicates that penetration of the onion layer by the hydrolyzed suppressor brings the enzymatic radiation of the layer to an end but does not suppress its degradational radiation. During reimmersion of the layer in the suppressor solution enzymatic radiation is also absent. Subsequent recooling again causes the appearance of degradational radiation.

These facts lead us to conclude that large quanta of energy necessary for the production of degradational radiation may be produced not only on account of the energy of recombination of free radicals or atoms, arising rarely in the course of enzymatic processes, but also as a result of the summation of small quanta, possessing far less energy, for the direct formation of photons of degradational radiation.

SUMMARY

The duration of degradational radiation was studied, as well as the property of molecular constellations to accumulate energy and to sum up its low quantity. It was established that considerable quanta of energy required for the appearance of degradational radiation may appear not only due to energy of recombination of the free radicals of atoms (which is seldom and occurs in enzymatic processes), but also as a result of summation of the quanta which possess too little energy to form the photons of degradational radiation directly.

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REGENERATION OF SUPERNUMERARY LIMBS IN TADPOLES

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In tadpoles and adult specimens of the lake frog (Rana ridibunda Pall.) from certain natural reservoirs situated in the foothills of the Zailiiskii Ala-Tau we often observed supernumerary hind limbs [2, 3]. Increase in the number of limb buds occurred mainly on the right side of the body and became evident visually long before metamorphosis, at an early stage of larval development when the hind limbs appeared as oval, undifferentiated buds. Evidently the factors causing the appearance of supernumerary limbs exert their influence at a period of primary concentration of cellular material, since later may be observed fully formed buds of several limbs in a varying degree of isolation (Fig. 1).

In their external features and microscopic structure the buds of the principal and supplementary limbs are hardly distinguishable from each other, and it is often difficult to decide from which bud the principal and from which the supplementary limb will develop. In many cases the rudimentary principal right limb was identified by its symmetrical position in relation to the left, and the buds of the supernumerary limbs may be identified by their unusual situation.

In early stages of development we observed, besides a large number of cases of two or several isolated appendages, cases in which the principal and supernumerary limbs were joined together for a considerable part of their length (see Fig. 1, b, c and d). At the moment of appearance of the first outward signs of the onset of differentiation of the indentations of the forming digits, a definite spatial relationship between the combined rudiments of the limbs can already be observed. This same relationship is basically retained in the subsequent period of development, although the rate and degree of differentiation of different limbs may vary considerably. With the appearance of visible articulation in the limb at its main sections there often takes place inhibition of growth and development of the supernumerary limb both in cases of isolated supernumerary appendages and when combined with the appendage of a principal limb. Inhibition of the subsequent development of the supernumerary limb at this stage and slightly earlier is often combined with marked depigmentation of its skin covering.

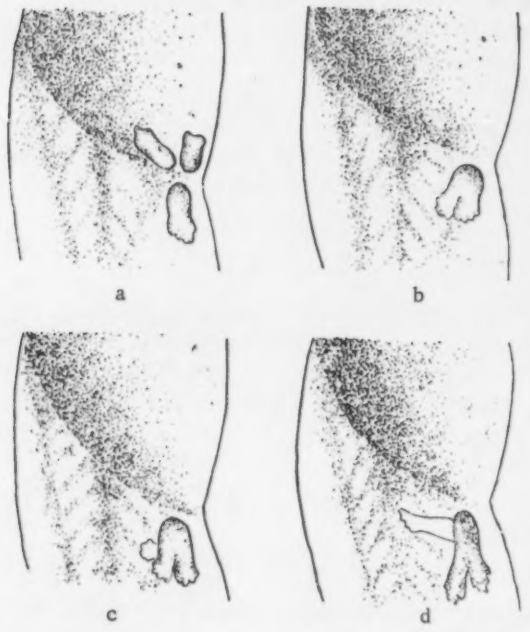

Fig. 1. Different cases (a, b, c, d) of relationship between supernumerary and principal limbs on the right side of the body at the stage of the first signs of external differentiation.

The supernumerary limb, as a rule, grows together with the principal limb, and the following relationships between them apply. Most commonly both limbs are disposed with their ventral surfaces side by side, with fan-like arrangement of the digits of the two feet; in this form the skin which is common to the combined limbs loses its polarity in pigmentation and acquires a homogeneous coloring, characteristic of the dorsal surface. In another type the supplementary limb is situated with its ventral surface on the dorsal aspect of the principal limb, while preserving the typical dorsoventral polarity in pigmentation of the skin of both limbs. The degree of combined growth of the two limbs may differ;

most commonly the limbs are joined only in the thigh region. The trophic conditions appear to be more favorable in the first type of combined growth, and the supernumerary limb often develops without any essential abnormality.

Our observations and experiments were made on anomalous tadpoles of lake frogs caught under natural conditions and continuing to develop in the laboratory. In tadpoles kept in large aquaria we studied the regeneration of the principal and supernumerary limbs. We were concerned with the problems of the extent to which the relationships formed during embryogenesis between the principal and supernumerary limb buds are stable and may be reproduced vegetatively in the process of reparative regeneration; to what extent does regeneration of supplementary limbs proceed typically, if we take into consideration our previous findings that these limbs are devoid of any motor innervation [4].

EXPERIMENTAL METHOD

Experiments lasting for a period of 3 years were performed on 62 anomalous tadpoles, of which 44 had isolated supernumerary limbs and in 18 they were joined to the principal limb bud. The number of regenerating limbs was twice this figure, since in addition to regeneration of the supernumeray limb, restoration of the principal limb was also studied. For the experiment we took tadpoles with initial signs of differentiation of the hind limbs, and not yet having lost their regenerative capacity [1, 5, 6]. The difficulty of obtaining the necessary material naturally did not permit us to perform all the experiments simultaneously. For this reason the experiments were performed at different times on single specimens as they were caught. Our annual observations were mainly made in July, just at the time when the metamorphosis of the bulk of the larvae of the lake frog is over and anomalous tadpoles can be found, developing evidently from spawn of later layings. Four large anomalous larvae, hibernating in the reservoir and found at an early stage of development were taken for an experiment at the end of April.

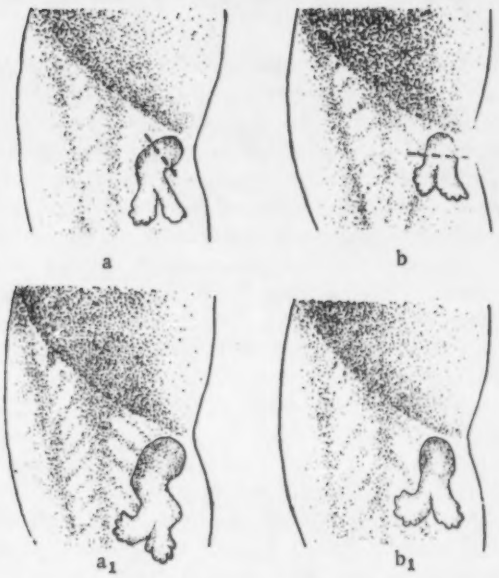

Fig. 2. Original state of the limbs before amputation of the distal portions (a, b); the level of amputation is shown by a broken line and the newly regenerating limbs in (a_1, b_1). The tadpoles were exposed to a low temperature for a short time (5 days).

The operative technic was the usual one. With a fine blade the principal and supernumerary limbs of the right side of the body were amputated perpendicularly to the main axis. The amputation was always performed at as proximal a level as possible (Fig. 2, a, b and Fig. 3, top series). The regenerating limbs were periodically drawn and a part of the material was prepared histologically.

In some experiments by Tornier [7], amputation of the limb buds which were accompanied by formation of supernumerary limbs was performed in line with the main axis, in anteroposterior or dorsoventral directions. It is relevant to remember that the power of regulated regeneration of the developing hind limb of the frog is retained for quite a long time during the larval period.

EXPERIMENTAL RESULTS

We may examine briefly the results of regeneration of isolated supernumerary limbs. Regeneration of these limbs took place synchronously with the restoration of the principal limbs amputated at the same time. They often finished up as perfectly restored limbs, with the formation of a normally formed foot; atypical regeneration consisted of a reduction in the number of digits, and diminution in size and disturbance of the relationship between the main sections of the limb. When the tadpoles were kept under optimal conditions in large aquaria with an abundance of food, the number of atypically regenerating principal limbs did not exceed 10%, while in the case of supernumerary limbs the figure was over 70%. A characteristic feature was that the atypical development of

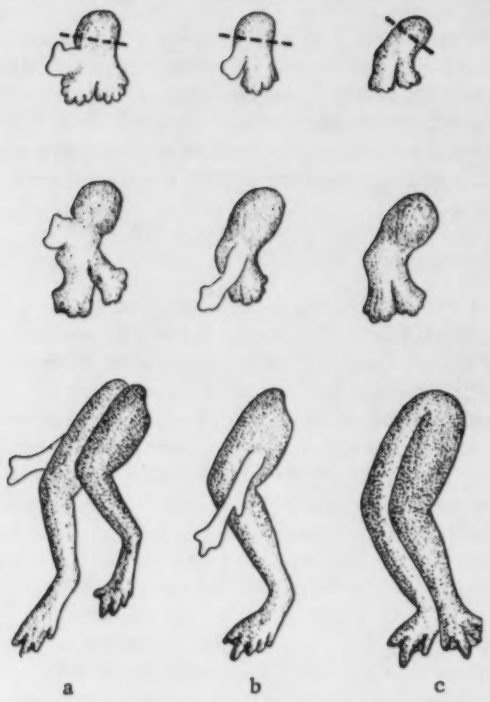

Fig. 3. Young fused limbs at the moment of amputation (top row), structure of the regenerating limbs at an early stage (middle row) and the same limbs after completion of differentiation into the typical organ at the stage of incipient functioning (bottom row).

a regenerating limb of the supernumerary type appeared and grew more pronounced at an age after the formation of a relatively normally formed foot on the young limb and the creation of the indentations of the rudimentary digits.

The most interesting findings were concerning regeneration of fused limbs, and were obtained in 18 anomalous tadpoles. During the process of regeneration the same relationships between the principal and supernumerary limbs which applied at the moment of amputation were repeated without any substantial difference. The young regenerating limbs repeated with only negligible variation the relationships which existed between the limb buds at the time of amputation. In one case we observed suppression of regeneration of a supernumerary limb with a reduction in the number of digits formed.

In another experiment performed in the following summer on different tadpoles with approximately the same anomalies, the phenomenon of repetition during regeneration of the pre-existing relationships was again confirmed (Fig. 3). Observations on the development of regenerating limbs continued for over a month, which enabled the later stages of formation of the regenerated limb to be traced. In the great majority of cases the regenerating supernumerary limbs lagged behind very considerably in their differentiation, which was combined with a partial reduction in the digits which had begun to form (see Fig. 3, a, b, c). These regenerating limbs were depigmented and, as histological examination showed, muscular tissue was absent, together with nerve fibers and vessels. In other cases the supernumerary limb regenerated without any significant abnormality, although its general growth was retarded; in these cases one of the digits in the regenerating foot of the principal limb was observed to be reduced. Where there was considerable fusion of the young buds, the regenerating combined limbs were well developed and were practically indistinguishable from each other (see Fig. 3, c).

Thus in the process of regeneration, i. e., of vegetative reproduction, there takes place a quite faithful repetition of the relationships which governed the principal and supernumerary limb buds in embryogenesis. In the process of repair we observed that same characteristic delay in differentiation which had already been noted in the development of the limbs of other anomalous specimens which had not been amputated. We consider that the absence of efferent innervation in supernumerary limbs, which we observed in conditions where there was a natural increase in the number of limb buds, could influence the nutrition and the character of the subsequent differentiation of the regenerating limbs. This did not show a negative influence in the case of the close anatomical and trophic union of two forming or regenerating limbs.

We may note in conclusion that if anomalous tadpoles are kept for two days before amputation and for 3 days after amputation in a vessel of cold water (4-6°C) they later form regenerating limbs which most clearly reproduce the previously existing relationship between the principal and supernumerary limbs. This is shown in Fig. 2; here, in contrast to the cases shown in Fig. 3, no disturbance of differentiation of the regenerating supernumerary limbs was observed, including those which develop at later stages of development. In the 4 hibernating anomalous tadpoles the regenerating limbs illustrated very clearly the stability of the morphological relationship of the principal and supernumerary limbs. Consequently, a low temperature promotes a more stable reproduction of the whole range of morphological signs of duplicate limbs and their relatively greater differentiation at a later stage of development.

SUMMARY

Amputation of supernumerary hind limbs was performed in 62 tadpoles (Rana ridibunda Pall). Regeneration of the principal and supernumerary limbs takes place with recurrence of the topographic-morphological relationships formed during the embryonic stage of development. Atypical formation of distal portions of the newly formed limbs takes place with appearance of external differentiation. Low temperature promotes a more stable recurrence of morphological signs of duplicate limbs by the regenerates.

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A TRIAL OF GROWTH HORMONE PREPARATIONS IN RATS WHOSE THYROID FUNCTION HAS BEEN BLOCKED WITH METHYLTHIOURACIL

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The discovery of Evans and Long of the growth hormone in 1921 prompted numerous attempts to isolate this substance in the pure form, which was achieved only in 1945 by the production of a highly purified preparation in the form of an individual protein [6]. However, the search for new, simpler and more convenient methods of biological testing of this hormone remains a problem of great importance; the most widely used tests, requiring preliminary hypophysectomy, are too complicated to be used as standard methods. Ia. M. Kabak and E. B. Pavlova [3] proposed the use of rats treated with methylthiouracil as test animals for growth hormone preparations, showing that blocking the thyroid gland function (as also by thyroidectomy) leads to cessation of formation of the growth hormone in the anterior lobe of the pituitary gland: the animals stop gaining weight, and in the anterior lobe of the pituitary the acidophil cells [2] which are generally held to produce the growth factor disappear; then, as a result of administration of growth hormone, growth is restored, while in the pituitary the histological changes do not take place.

* In Russian.

** See English translation.

This present work was undertaken as a development of these investigations with the following aims: 1) to confirm, by using one of the most specific indices of growth – the width of the cartilaginous growth plate of the proximal epiphysis of the tibia – that in rats receiving methylthiouracil the gain in weight which occurs as a result of the growth hormone is in fact due to true growth; 2) to investigate this test in rats with blocked thyroid gland function in greater detail, in particular to discover any relation between the dose of the pituitary preparation and the effect produced.

Replacement of hypophysectomy by blockage of thyroid function, in our opinion, not only simplifies the test procedure but has other advantages over hypophysectomy: absence of surgical trauma; exclusion not only of the growth factor of the pituitary but also of thyroid gland function which is especially important in the case of an inadequately purified preparation, since synergism between the growth hormone and the thyreotropic hormone (in the presence of a functioning thyroid gland) may result in an incorrect assessment of the activity of the growth hormone preparation [7].

Some of the findings presented here formed the subject of a lecture given to the All-Union Society of Endocrinologists [1].

EXPERIMENTAL METHOD

In this experiment rats of both sexes were used, the majority weighing 40-60 g. The diet of the animals consisted of porridge, to which was added fish oil, milk and finely chopped meat. Methylthiouracil (MTU) was mixed with this diet in a dose of 20-30 mg per rat (depending on the weight of the animal) per day. The amount of food given was roughly that consumed in the course of 24 hrs. In addition the animals received carrots or greens. The rats were kept on this diet for 35-62 days; every 5-7 days they were weighed and, after they had ceased to gain in weight, in addition to the continued administration of MTU they were given subcutaneous injections twice daily containing a preparation of pituitary growth hormone (PGH) prepared in accordance with the description by Ia. M. Kabak and E. B. Pavlova [3]. Besides the body weight the index of growth was the width of the epiphyseal cartilage of the proximal end of the tibia [6].

The animals were killed 24 hrs after the last injection, the tibia removed and treated by a method described by Greenspan et al. [5]: the tibia was cleared of soft tissues, divided in the median sagittal plane, and the halves of the bone either stained directly or first fixed in 10% neutral formalin. The staining procedure was as follows: the halves of the bone were immersed for 30 min in water, transferred into acetone for a minimum of 1 hr, and again washed in water for 30 min. Next they were placed for 1-2.5 min in a freshly prepared 2% solution of silver nitrate, washed in water and, without being taken out of the water, exposed to bright light until the calcified part had acquired a dark brown color. Afterwards the bone was immersed for 25-30 sec in a 10% solution of sodium thiosulfate and then rinsed in running water for 30 min. The width of the epiphyseal cartilage was measured under the low power of the microscope by means of an ocular micrometer. We took the average figures of 5-6 estimations of the transverse section of the cartilage.

EXPERIMENTAL RESULTS

We divided 23 male rats which received MTU for 62 days into 3 groups: one group acted as a control, the second was injected daily with 15 mg PGH per rat for 5 days, the third – 15 mg PGH per rat for 13 days. The results of the experiment are shown in Table 1.

As can be seen from Table 1, the rats receiving PGH injections for 5 days gained 12.5% of their original weight, and the mean width of their epiphyseal cartilage was 211% of that of the controls. Rats receiving PGH for 13 days gained 19.4% of their initial weight, and the mean width of their epiphyseal cartilage was 312% of that of the controls. The weight of the control rats receiving MTU was practically unchanged during this period.

We carried out a further trial of various doses of PGH. It was important to establish if the reaction of the epiphyseal cartilage of the tibia in these rats receiving MTU to PGH was quantitative in character. Table 2 answers this question in the affirmative, although variations are seen, connected with their individual characteristics. Altogether 5 experiments were performed on 108 rats of both sexes and receiving MTU for different periods of time.

TABLE 1

Changes in the Body Weight and the Width of the Epiphyseal Cartilage of the Tibia in Rats Receiving MTU After Injection of PGH (mean values)

Effect of	No. of animals	Body weight in g		Change in body weight		Width of epiphyseal cartilage	
		before injection of prep.	before death	in g	in %	in μ	in % of control
MTU (control)	7	85.8	86.2	+0.4	0.4	113	100
MTU + 15 mg PGH for 5 days	8	77.8	87.6	+9.8	12.5	239	211
MTU + 15 mg PGH for 13 days	8	92.5	110.5	+18.0	19.4	353	312

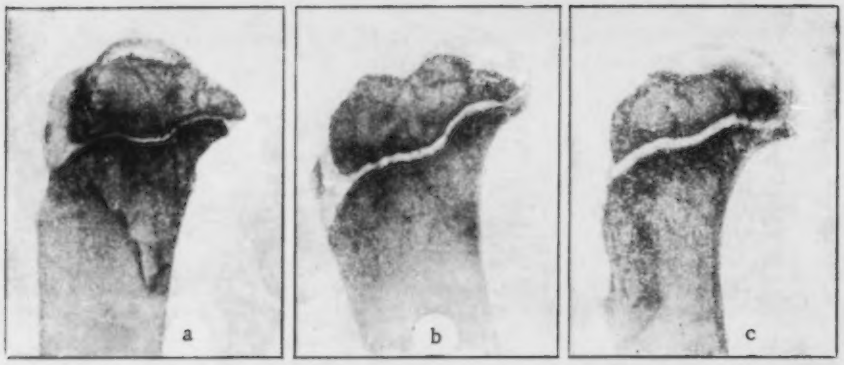
TABLE 2

Changes in the Width of the Tibial Cartilage of Experimental Rats After Injection of Different Doses of PGH (mean values)

Expt. no.	No. of animals in group	Dose of PGH (in mg)	Period of action of MTU in days	Width of tibial cartilage	
				in μ	in % of control
1	6	—		84	100
	5	0.5	55	116	138
	5	1.0		116	138
	5	2.0		129	153
	5	15.0		148	176
2	6	—	57	105	100
	6	2.0		189	180
	6	5.0		185	176
	7	15.0		213	208
3	5	—		110	100
	5	5.0	46	185	168
	5	10.0		191	174
	4	15.0		217	200
4	5	—	41	144	100
	5	5.0		202	140
	5	60.0		217	151
5	5	—		157	100
	6	7.5	35	234	149
	6	15.0		234	149
	6	30.0		239	150*

Before administration of the preparation was started the animals were divided into groups so that each group was made up as far as possible from males and females; one of these groups acted as a control in each experiment and received no other preparation than MTU; the rats of the other groups received two daily injections of the following doses of PGH: 0.5, 1, 2, 5, 7.5, 10, 15, 30 and 60 mg. Injections were give for a period of 4 days.

On the 5th day the rats were killed (in view of the short period of administration of the preparation these rats did not show any increase in weight).



Longitudinal sections of the proximal ends of the tibia of rats fed with MTU, stained with silver nitrate. a) Control (no PGH); b) PGH in a dose of 5 mg; c) PGH in a dose of 15 mg.

As may be seen in Table 2, a dose of 15 mg of PGH caused the most reaction in the cartilaginous plate, which increased in width roughly twice as much as that of the control (experiments 1, 2, 3). The least dose used – 0.5 mg – was also found to be active, giving a 38% increase in the width of the cartilage (experiment 1).

After feeding on MTU for 35 days (experiment 5) the width of the cartilaginous plate in the control rats was 157μ, and in this group the reaction of the cartilage was the same to different doses of PGH. With a more prolonged action of MTU – 41 days – (experiment 4), 46 days (experiment 3), 55 days (experiment 1), 57 days (experiment 2) – the width of the cartilage in the control animals varied from 144 to 84μ, while the reaction of the cartilage to PGH in all these experiments was quantitative in character, admittedly shown only very weakly in experiment 4, but well marked in experiment 3 (see figure), i. e., on the 46th day of action of MTU.

This investigation thus shows that rats kept in practically a state of athyreosis, do not form endogenous growth hormone; the width of the cartilaginous plate of the tibia, as shown above, is 84-157 μ, while in hypophysectomized rats, according to Evans and his co-workers [5], it is about 160 μ. The cartilaginous plate in the tibia of rats with blocked thyroid gland function was found to be sufficiently sensitive to growth hormone injected for a period of 4 days, and consequently this feature in these animals may be used as an index in the testing of the activity of the preparation.

SUMMARY

It was proved by the tibial test that the endogenic growth hormone is not produced in rats with blocked function of the thyroid gland. It was demonstrated that tibial epiphyseal cartilage is sufficiently sensitive to the corresponding preparations.

Preparations of growth hormone may be tested on rats in which the function of the thyroid gland is blocked by administration of methylthiouracil. This method is more convenient than the one used formerly. The latter was based on utilization of hypophysectomized rats.

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METHODS

RECORDING THE ACTION POTENTIALS OF NERVES IN AN UNSCREENED ROOM

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Until recently the only suitable method of protection against grid interference during work with weak electric signals such as nerve action potentials was careful screening of the room or even of the animal itself. However, working in a screened chamber, or placing the animal in a screened cage are both connected with great difficulty and inconvenience. This is an obstacle to the wide introduction of electrophysiological methods of investigation into practice. Recently a number of attempts have been made to construct special bioamplifiers and a method of working which would enable action potentials of an animal to be recorded in ordinary laboratory conditions without screening the object.

We set out to make a careful investigation of this problem and to determine what limits of sensitivity could be reached by recording nerve action potentials in an unscreened room. We divided the solution of the problem into 2 stages: 1) the construction of a bioamplifier possessing the highest possible coefficient of rejection, and 2) the development of a method of picking up the potentials, the selection of the optimal construction of the electrodes of the earthing system and so on.

Biological amplifiers, as a rule, are made according to a symmetrical circuit [1, 2, 4, 5, 8]. Each cascade contains 2 valves with separate anodic resistances of equal size and one general resistance R_k in the cathode circuit of both valves. The action potentials are picked up bipolarly and applied to the grids of two symmetrical valves in the first cascade. An amplified potential is taken from between the anodes of these valves and, as also in the opposite phase, is applied to the grid of the second symmetrical cascade and so on.

The main feature of the working of such an amplifier is that for an action potential which falls first on one valve of the input cascade and then on the other (as the wave of excitation moves along the nerve), the amplifying properties of the circuit are used to their full extent. The presence of the common, and usually high, cathode resistance R_k in this case does not lead to diminished amplification (there is no negative feedback). If, however, 2 electric signals, perfectly equal in amplitude, form and phase, reach the grids of both valves in the input cascade at the same time, then the resistance R_k will act as a negative feedback resistance and the amplification of this synphasic signal will be greatly diminished. Since grid interference is applied equally to both electrodes it will be weakened in comparison with the recorded potential of bioelectrical origin. This phenomenon is called rejection, and the ratio between the coefficient of amplification of the recorded antiphasic potential to the coefficient of amplification (in practice often weakening) of the synphasic potential, calculated for the amplifier as a whole, is called the coefficient of rejection.

As follows from the theory of the symmetrical amplifier [1, 2, 4, 5, 6, 8], the coefficient of rejection of such an amplifier is higher, the greater the magnitude of the common cathode resistance R_k and the higher the coefficient of amplification μ of the valves incorporated in it. The development of the amplifier circuit has therefore gone in the direction of maximum increase of both these parameters. Its final form is shown in Fig. 1.

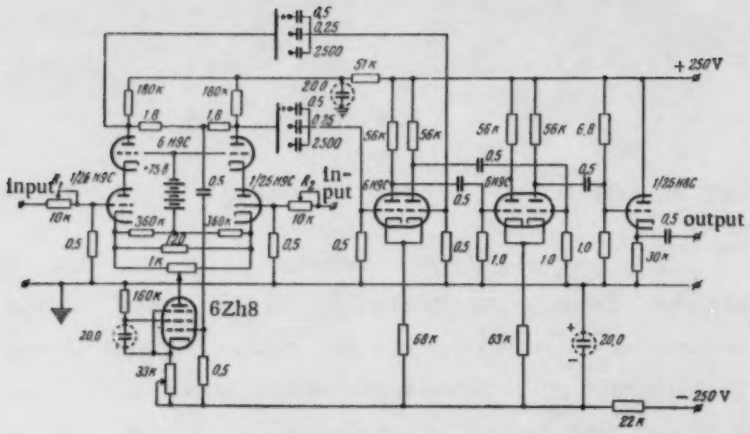

Fig. 1. Circuit showing the principle of the preamplifier.

As the common cathode resistance of the valves of the first cascade we used a pentode 6Zh8. This usage of the high dynamic resistance of a pentode has become a usual feature of the design of biological amplifiers in recent years [3, 5, 6, 8]. The dynamic resistance of the pentode is also increased on account of the negative feedback through resistances included in its cathode circuit.

Pentodes possess high intrinsic coefficients of amplification μ. For this reason some workers [5, 8] and foreign firms (for example, the American firm of Grass) use pentodes as the input valves of the bioamplifier. This solution does not seem justifiable to us, since pentodes differ from triodes in their higher noise level, which restricts the sensitivity of modern bioamplifiers.

In our amplifier, in place of each input valve of the symmetrical cascade we used a series combination of 2 triodes, the cathode of the second triode being directly connected to the anode of the first, the grid of which receives the potential to be amplified. The grids of the second triodes are connected together and their potential is relatively midway between the very close potentials of the cathodes of the first triodes, and is maintained constant by means of a dry battery. The amplified potential is obtained between the anodes of the second triodes, with which anode loads are included in series. A similar design including triodes was investigated by Klein [6]. He showed that such a combination of triodes possesses a very high coefficient of amplification, approximately equal to the product of the coefficients of amplification of each triode, and in this sense equivalent to a pentode; on the other hand it is distinguished by the characteristic low sound level of the triode [7]. Finally, following Richards [8], we led the negative feedback from the midpoint between the anodes of the second triodes to the controlling grid of the pentode, used as a cathode load. This lead profoundly lowers the change in the common level of anode potentials of both halves of the cascade during feeding of synphasic signals into its input; in this way the effect of the different dynamic characteristics of the valves is minimized and the rejection increased.

The second and third cascades of the amplifier are constructed according to the usual symmetrical design; the output signal is taken from the cathode repeater. Thermionic emission in the triodes of the first cascade is maintained by a low tension direct current (about 3 v). The second cascade is also supplied with direct current of normal tension. The voltages of +250 and −250 v are stabilized by electronic stabilized by electronic stabilizers.

In this design we obtained a value of more than one million for the coefficient of rejection, which was clearly adequate for carrying out the second part of the investigation.

In its construction the apparatus is produced in the form of easily transportable sections — a two-channel preamplifier section and a power section; neither is larger than the usual electric record player. The apparatus is wholly worked from the alternating current mains.

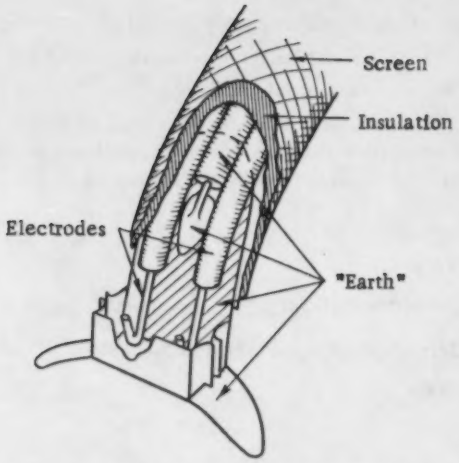

Fig. 2. Construction of the electrode for testing the action potentials of a nerve (magnified).

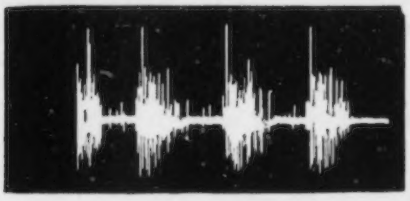

Fig. 3. Oscillogram of impulses from the depressor nerve of the cat taken without any form of screening of the object.

The preamplifier has a coefficient of amplification of 20,000 throughout a wide range of working frequencies of 20 cps to 8 kilocycles, and is designed for working with an industrial double-beam OK-17M oscillograph. The amplification is controlled in the oscillograph itself. The recordings on the oscillograph screen are transferred to a cinematograph film.

In developing a method of picking up the biopotentials we became convinced that earthing the animal itself is unnecessary and even harmful, since it creates the possibility of production by a magnetic field of an alternating current in the "loop" animal – electrode – "earth" of the amplifier – "earth" of the animal. We earth the nerve directly at its point of contact with the body of the animal, i. e., almost at the testing electrode itself. If during testing the nerve is not divided or if its divided end is in contact with the animal's body (even if only through a moist ligature), it is earthed on both sides. Earthing is done through a "wing" of a brass tin-covered box inside which and insulated from it are placed the platinum hooks of the electrodes (Fig. 2).

Emerging through lateral slits in the box, the nerve is placed on the "wing" and pressed against it by a cotton swab soaked in Ringer's solution. The box itself is connected by means of a third lead, which must be bound together with the two leads which come from the electrodes, to the "earth" inside the amplifier itself. All 3 leads are contained in a screened, flexible cover which leads almost to the electrodes, and is also earthed at one point only, close to the amplifier. This prevents the development of magnetic loops in the testing electrodes and the screen itself. After the nerve has been placed on the electrodes the box may be covered with vaseline. On account of the narrowness of the vertical slits the vaseline does not escape.

The method of picking up the potentials just described, together with the high rejection of the amplifier, enabled us to solve the problem of recording the action potentials of nerves in an animal without the use of a screen. As an illustration in Fig. 3 is shown the oscillogram of impulses taken from the depressor nerve of the cat. The power feed, transformer, illuminating lamp and other items were situated a few centimeters from the animal. The experimenter was able to manipulate the animal with his hands. In spite of this, the manipulations did not exceed the level of sound production. This level in Fig. 3 corresponds approximately to 20 μv "from peak to peak" (with a frequency range of 8 kilocycles).

In the course of further investigation of the apparatus we found that the figure of rejection obtained can be lowered more than ten times without interfering with its working. This was achieved by introducing into the input circuits of the apparatus balanced resistances R_1 and R_2 (controlled by the same handle). The importance of these resistances is that they equalize the amplitudes of the impulses reaching the grids of the input cascade of the amplifier from the electrodes, which are almost always unequal. Naturally this equalization must be carried out with the highest precision, and in consequence the preservation of such a high rejection as we had at first loses its purpose. Nevertheless we look upon the work of creating an amplifier with a very high coefficient of rejection as showing great promise, so that in the very near future it can evidently be expected that the level of sensitivity of bioamplifier will be further raised.

SUMMARY

By employing in each half of the symmetrical input cascade of the amplifier a series combination of two triodes and a pentode as a total cathode load with a deep inverse feedback – it becomes possible to bring the rejection factor of such an amplifier up to one million (without the matching of tubes).

A portable double-channel preamplifier using such a circuit has been developed for work with an industrial double-beam oscillograph. With the aid of this preamplifier and electrodes of special construction it was possible to register the potential of nerve action of animals in short-term experiments without screening the room and object in usual laboratory conditions.

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A METHOD OF STUDYING GASTRIC AND DUODENAL SECRETION AT THE SAME TIME**

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(Received April 22, 1957. Presented by Active Member Acad. Med. Sci. USSR, N. A. Rozhanskii***)

I. P. Pavlov (1897, 1902) repeatedly drew attention to the development of methods which would enable the simultaneous observation of the work of several digestive glands in the same normal animal.

The performance of this task is often beset by difficulties: on the one hand it is necessary to obtain, measure and test the juice from each gland separately, and on the other hand, the juices must enter the digestive tract in the same quantities as were secreted by the glands both outside and during the experiment, otherwise the normal course of the digestive process would be disturbed.

* In Russian.

** Delivered (with a demonstration on a dog) at the meeting of the Riazan section of the Society of Physiologists on December 27, 1955.

*** Deceased.

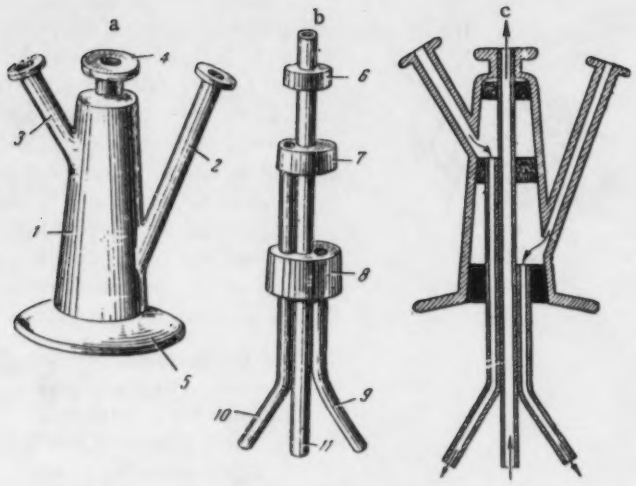

Fig. 1. a) Fistula tube; b) the "heart" of the apparatus; c) the fistula tube with the "heart" in situ (in section); 1) body of fistula tube; 2) first branch of fistula tube (for collection of gastric juice); 3) second branch of fistula tube (for collection of pancreatic juice); 4) upper ring of fistula tube, inserted in the duodenum; 5) lower ring of the fistula tube brought out onto the surface of the animal; 6) ring of the "heart," closing the upper orifice of the fistula tube; 7) ring of the "heart," closing the fistula tube between its first and second branches; 8) ring of the "heart," closing the lower part of the fistula tube; 9) drainage tube of the "heart" for collection of gastric juice; 10) drainage tube of the "heart" for collection of pancreatic juice; 11) central tube of the "heart" for returning to the intestine the digestive juices and for infusion of test substances.

It is particularly difficult to study simultaneously the work of the two most important and closely interconnected digestive organs – the stomach and the pancreas.

The difficulty of preserving the life of animals with an isolated stomach or with a pancreatic fistula, and of looking after them, is well known. These difficulties may be primarily responsible for the absence of work dealing with the study of the secretion of gastric and pancreatic juice by experiments on the same animal. We decided to attempt to overcome these difficulties.

In order to obtain gastric and pancreatic juice simultaneously we used the fistula tube suggested by one of us [4] (Fig. 1, a, b, c). In accordance with its new functions the construction of the tube was slightly modified.

The diameter of the body of the tube (1) was enlarged, and the orifices of its branches (2 and 3) and also of the drainage tubes of the "heart" (9 and 10) were widened.

The fistula tube is made from the plastic AKR-7, used for dentures, and it can be made in any dental prosthetic workshop. Tubes of this material are sufficiently firm, light and resistant to acids and alkalies. They differ from the heavy, metal tubes by not causing ulceration.

The "heart" is inserted in the fistula tube only at the time of the experiment. It is turned from stainless steel. The drainage tubes (9 and 10) are made either from stainless steel (which is better but more difficult) or from German silver. The central tube of the "heart" (11) is turned in one piece with its rings (6, 7, 8). It is very important that all three metal rings of the "heart" (6, 7, 8) should be well ground to the body of the tube (1). With poor grinding it is possible for juice to leak from one compartment of the tube to another. For hermetic sealing of the rings of the "heart" (6, 7, 8) they are smeared with a thin film of vaseline before being inserted in the fistula tube.

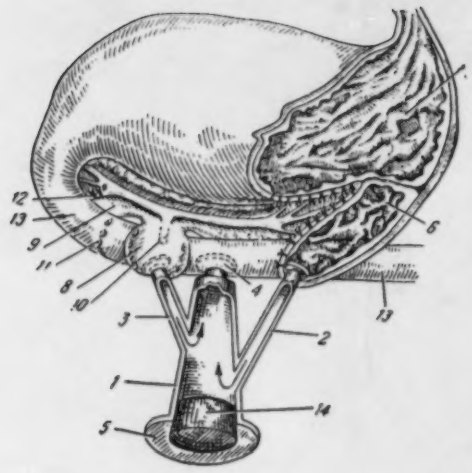

Fig. 2. Diagram showing the disposition of the fistula tube in the stomach and duodenum. Part of the stomach and the fistula tube are shown in section. The "outside the experiment" position is shown, when the fistula tube is closed with a rubber stopper. 1) Fistula tube; 2,3) see Fig. 1; 4) upper ring of the tube in the duodenum; 5) lower ring of the tube brought out onto the surface of the abdomen of the dog; 6) isolated stomach pouch (in section); 7) stomach; 8) isolated duodenal pouch for collection of pancreatic juice; 9) pancreas; 10) main pancreatic duct; 11) intestinal anastomosis; 12) accessory duct of the pancreas, ligated and divided; 13) duodenum; 14) rubber stopper closing fistula tube.

The operations on the gastrointestinal tract required in this method do not call for description, since they are carried out by the usual routine for preparation of an isolated gastric pouch [3, 6] and duodenal pouch [1, 2, 5]. It needs pointing out only that in contrast to the usual method the end of the isolated gastric pouch is not exteriorized to the surface of the animal but the branch of the fistula tube is fixed into it by means of a purse-string suture (Fig. 2, 2). Together with the fistula tube, the isolated gastric pouch is completely contained in the peritoneal cavity. The whole operation lasts 2-2.5 hrs if the surgeon is sufficiently well prepared.

Outside the experiment the fistula tube is closed by an ordinary rubber stopper. In this case the gastric juice from the isolated stomach pouch, and also the pancreatic juice are directed into the duodenum (Fig. 2). The digestive process proceeds normally outside the experiment.

At the time of the experiment when the "heart" is in position inside the fistula tube (Fig. 1), gastric juice from the isolated stomach pouch flows along the branch of the fistula tube (2) and also along the drainage tube of the "heart" (9) into one test tube, and the pancreatic juice from the isolated duodenal pouch along the branch of the fistula tube (3) and the drainage tube of the "heart" (10) into another test tube.

Every 5 min (for gastric juice — usually after 30 min) both juices, after measurement and taking of samples for analysis, are returned through a rubber tube and funnel and the central tube of the "heart" (11) into the duodenum.

Therefore, during the experiment also, both juices enter the digestive tract in almost their full amounts and at the right time, and digestion proceeds normally.

The great advantage of the method described is the tremendous simplification of the care of the dogs after operation. It is not necessary after giving the dog a meal to keep it for a long time, outside the experiment, in a stand as has to be done with dogs with isolated gastric pouches. No longer is it necessary to carry out daily washing of the abdomen and limbs of the animal, and to watch carefully for signs of inflammatory or ulcerative lesions of the skin of the abdominal wall due to the gastric and pancreatic juices, or to administer soda to the dogs daily to replace the loss of alkali. In practice the care of the dogs is no more complicated than in the case of animals with a Basov gastric fistula.

If the operation is carried out with sufficient care and leakage of juice along the track of the fistula can be prevented, the animals may live for a long time in good health and well-being, and can be used not only for research purposes but also for educational purposes.

SUMMARY

A method is described which allows obtaining of gastric juice from an isolated stomach pouch simultaneously with pancreatic secretion and then after measuring their quantity to return them into the duodenum. During the time between the experiments both juices enter the intestine.

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EXPERIMENTAL HETEROTRANSFUSIONAL SHOCK IN WHITE RATS ACCOMPANIED BY ACUTE PULMONARY EDEMA

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In the practice of medicine, cases are observed of death of patients after transfusion of incompatible blood as a result of acute edema of the lungs [2, 3, 7].

The pathological findings show that pulmonary edema in patients dying from blood transfusional shock [4] or other forms of shock [8] is very common. However, until our report [5] we could not find any reference in the literature to a study of the pathogenesis of pulmonary edema due to transfusion of blood from another species of animal. Accordingly we transfused foreign blood into various animals. In particular we injected white rats with blood from cattle, dogs, rabbits, sheep, goats, geese, pigeons and man. Preliminary experiments showed that blood from oxen, cows and calves causes shock which is accompanied by acute pulmonary edema.

EXPERIMENTAL METHOD

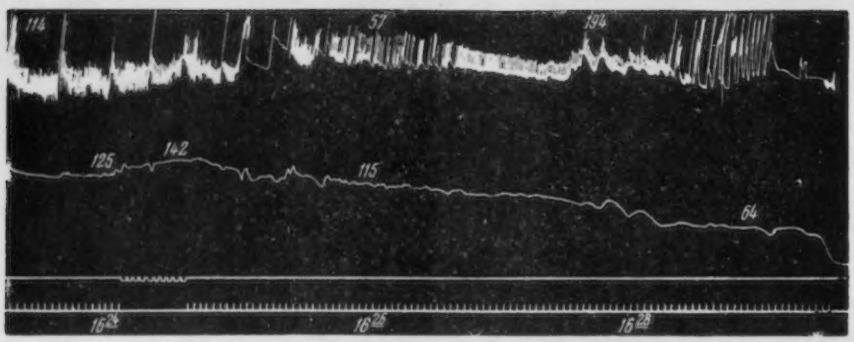
In the experiment we used white rats of both sexes, weighing from 52 to 329 g. Ox** blood was obtained from a slaughter house of the meat combine, observing aseptic precautions. Sodium citrate was used as an anticoagulant (0.3-0.5 g per 100 ml blood). As a rule the blood was used the day it was taken, after filtration through 6-8 layers of gauze. The blood was injected into the femoral vein of unanesthetized rats in a period of 30 sec. In some investigations we obtained tracings of the blood pressure in the carotid artery and of respiration. In a number of experiments we investigated variations in the size of the kidneys. The dead animals were examined post mortem and histological examinations were performed.

EXPERIMENTAL RESULTS

The first series of investigations consisted of 238 experiments in which rats were injected with ox blood in a dose of from 10 to 25 ml/kg; 223 of these experiments did not include kymographic recording. In 11 of these

* In Russian.

** In future, blood from bulls, cows and calves will be called ox blood.



Changes in respiration and arterial pressure during injection of a white rat with ox blood. Interpretation of the curves (from above downwards): respiratory movements (the figures indicate the number of respirations in one minute); arterial pressure (the figures indicate the height of the arterial pressure in mm Hg); zero line and marker of transfusion of ox blood in a dose of 15 ml/kg; time marker (3 sec). After death the lungs of the rat weighed 22.4 g.

223 experiments the rats had been injected 12-13 days earlier with ox blood; in 7 experiments heterogenous blood was injected (20 ml/kg) after preliminary bleeding (from 14.6 to 22.6 ml/kg); in 2 experiments the infusion was made into the femoral artery against the blood-flow; in 4 experiments the rats were injected with ox plasma instead of blood; in 7 experiments serum was injected.

Under the conditions of the experiments these variants did not show any essential difference from the typical picture of shock.

Fatal shock developed in 160 of the 238 animals. The animals survived for 1.4 min to 24 hrs after the infusion, the majority dying between 3 and 13.5 min afterwards.

The experiments showed that in order to produce fatal shock it was sufficient to inject the white rat with 10-25 ml/kg of ox blood, plasma or serum in a period of 30 sec.

As the experiments showed (Fig. 1), at the very beginning of the infusion of ox blood a rise in the blood pressure was observed by 16-32 mm Hg. Later, after .5 to 3.5 min, it fell in a fluctuating manner.

The marked rise in the arterial pressure during blood transfusion depends on the specific action of ox blood as a pathogenic stimulus and not on a nonspecific cold or volume effect of the fluid injected. This is shown by control experiments in which rats were injected with the same and larger doses of a 0.85% saline solution.

At necropsy of the rats which died from shock, in addition to slight congestive changes in the abdominal organs, severe lesions were observed in the lungs: they did not collapse and had the appearance of marble. The bronchi and trachea were filled with transudate containing from 4.78 to 7.32% of protein. If the so-called weight index of the lungs of normal rats, in our experience, varied from 4.2 to 10.4 (with an average of 6.4 in 28 animals), in the rats dying from shock the corresponding variations were from 10.2 to 38.2 (with an average of 23.7 in 96 animals). We may mention that there was pulmonary edema also in rats killed after development of a nonlethal shock.

Histological examinations of the experimental rats confirmed the presence of acute edema of the lungs and of certain dystrophic changes in the liver and kidneys.

It should be pointed out that workers [1, 6] who injected white rats with rabbits' blood found no significant pulmonary edema after death, but constantly observed changes in the form of subperitoneal pinpoint hemorrhages on the intestine [1]. We rarely found this feature, but constantly observed an intensive edema of the lungs.

SUMMARY

Intravenous injection to white rats of blood, plasma or serum of bulls, cows and calves (in the dose of 10-25 ml/kg body weight within 30 sec) brings about hemotransfusional shock which causes rapid death of animals. Development of acute hemorrhagic edema of the lungs is the characteristic sign of this shock.

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ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci.-Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

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